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STABLE CARBON AND NITROGEN ISOTOPE RATIOS IN THE SHALLOW-WATER CAPE HAKE, *MERLUCCIUS CAPENSIS* (CASTELNAU) AS INDICATORS OF TROPHIC POSITION AND DIET ON THE WEST AND SOUTH COASTS OF SOUTH AFRICA

by

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Dissertation presented to the Department of Zoology, University of Cape Town in fulfilment of the requirements for the degree of Master of Science.

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February 1993

DECLARATION

This dissertation reports the results of original research which I have carried out during 1991 and 1992 in the Marine Biology Research Institute, University of Cape Town. These results have not been previously submitted for a degree at this or any university. I collected the majority of the samples on four 2-3 week long cruises on board F.R.S. *Africana*. Supplementary samples were collected by Sea Fisheries Research Institute staff, whose assistance is fully acknowledged. All laboratory-based sample processing and mass specrometry was carried out by myself.

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ABSTRACT

$^{13}C/^{12}C$ and $^{15}N/^{14}N$ were used to indicate the trophic levels of the shallow-water Cape hake, *Merluccius capensis* (Castelnau) at three sites on the west coast of South Africa, and five sites on the south coast. Gut content analyses show only the very recent diet of hake, therefore stable carbon and nitrogen isotope ratios were used to show the longer-term diet, integrated over the turnover time of the muscle tissue and bone collagen analysed. $^{13}C/^{12}C$ is 1-2$^{o}/_{oo}$ higher in the tissues of a consumer than its diet (DeNiro and Epstein 1978), the difference in $^{15}N/^{14}N$ between a consumer and its food being 3-4$^{o}/_{oo}$ (DeNiro and Epstein 1981). Both $^{13}C/^{12}C$ and $^{15}N/^{14}N$ indicate trophic enrichment between hake muscle tissue and bone collagen, and the gut contents and prey, and show that small and large hake feed at different trophic levels, large hake tissues being slightly heavier in ^{13}C than small hake tissues, and containing 2-4$^{o}/_{oo}$ more ^{15}N than muscle tissue and bone collagen of small hake. A positive correlation is found between $^{15}N/^{14}N$ and increasing fish length. $^{15}N/^{14}N$ is a better indicator of the trophic position of hake, and shows two dietary pathways by which small hake feed on the south coast.

Variation in $^{13}C/^{12}C$ occurs with season in zooplankton samples and the muscle tissue and bone collagen of small hake on the west coast; the zooplankton samples contain more ^{13}C in summer, while the tissues of the small hake have higher levels of ^{13}C during winter. Seasonal variation in $^{13}C/^{12}C$ in the fish tissues and zooplankton may be due to greater biological productivity associated with upwelling on the west coast of South Africa during summer. No seasonal variation was found in $^{15}N/^{14}N$.

A significant difference in $^{15}N/^{14}N$ was found in hake muscle tissue and bone collagen between the west and south coasts, samples collected from the west coast containing more ^{15}N than the south coast samples. This is attributed to environmental and biological differences between the two coasts. The $^{13}C/^{12}C$ ratio was not significantly different between the west and south coasts.

Muscle tissue from hake sampled on the west coast had higher levels of ^{13}C than bone collagen, and ^{15}N was higher in the bone collagen than muscle tissue in all hake samples. This

is probably caused by the different amino acid composition of the two tissues, and variations in the stable isotope ratios of the individual amino acids.

It is concluded that stable carbon and nitrogen isotope ratios are useful to indicate trophic relationships in hake, and to estimate the long-term diet of the fish integrated over the turnover time of muscle tissue and bone collagen.

1. INTRODUCTION

1.1 The Cape Hake

Cape hakes are the dominant fish caught by demersal trawlers off the South African coast, constituting about 70-75% of the catches (Botha 1970, Crawford *et al.* 1989, Payne 1989). The largest hake fishery on the world is supported by the two hake species present off the South African coast (Botha 1980). *Merluccius paradoxus* (Franca) is found in water depths of 150-800m, and is dominant in the deep water of the west coast (Payne 1989, Payne and Punt 1993). *Merluccius capensis* (Castelnau) is found inshore of *M.paradoxus* (Payne *et al.* 1987), to a depth of 380m on the west coast, and is dominant on the south coast (Payne 1989). *M. capensis* has been found in water as shallow as 17m on the Agulhas Bank, some large individuals occurring down to 450m (Badenhorst and Smale 1991). Juvenile fish are found inshore of the adults of the species, fish size increasing with increasing depth (Payne *et al.* 1987). Until the 1960's it was assumed that only one hake species, *Merluccius capensis*, was present off the South African coast (van Eck 1969), the difference in fish size with depth being attributed to offshore-onshore migrations (Roux 1949). The two hake species are difficult to tell apart, morphological differences (eg. eye size, colouration, gill structure and body shape) being subtle (Payne 1989). Commercial catches make no distinction between the two species (Botha 1980), and they are managed together. Stocks of hake on the west and south coast are assessed separately within the statistical areas assigned by the International Commission for the Southeast Atlantic Fisheries (ICSEAF) (Payne 1989). In this study division 1.6 corresponds to the west coast area, and divisions 2.1 and 2.2 (which are managed together) correspond with the south coast area. The stocks may differ in growth rates and colouration with area (Payne 1989). This study concentrates on the shallow-water Cape hake, *Merluccius capensis.*

The feeding of hake has been documented by Assorov and Kalinina (1979), Botha (1980), Prénski (1989), Payne (1986) and Payne *et al.* (1987). These studies are based on gut content analyses, which show only the food consumed by the fish in the immediate past. Hake are opportunistic feeders, consuming available prey (Payne *et al.* 1987). Prey availability varies

seasonally and with geographic area (Payne *et al.* 1987). Small hake on the west coast feed mainly on crustaceans (Payne *et al.* 1987), particularly euphausiids (*Euphausia lucens*) which are dominant in the zooplankton on the west coast (Hutchings *et al.* 1991), amphipods (*Themisto gaudichaudi*), stomatopods (*Pterygosquilla armata capensis*) and decapods (*Funchalia woodwardi*). Small hake in the Cape Columbine area also feed on myctophid fish (Chlapowski 1977 in Payne *et al.* 1987), particularly the lanternfish *Lampanyctodes hectoris*. As hake increase in length, they incorporate more fish into their diets (Payne *et al.* 1987). Hake of both species constitute a major portion of the diet of *M. capensis*, as they are the most dominant fish found in their habitat (Botha 1980). Other fish in the diet of hake on the west coast include pelagic fish, and other demersal species (Payne *et al.* 1987).

According to Payne (1986), hake are more piscivorous on the south coast than on the west coast. Small hake appear to consume fish at an earlier age than on the west coast, possibly due to a scarcity of inshore planktonic crustaceans or more small fish species available for consumption (op.cit.) Other demersal fish appear to be less important as hake prey on the south coast, while pelagic fish constitute more of the hake diet than on the west coast (op.cit).

To date, hake feeding has been estimated only by intensive gut content analyses, which are subject to bias due to various digestion rates of different prey and feeding in the trawl net. Hake are the dominant catch in the demersal fishery. Some of the pelagic fish they consume, eg. anchovy (*Engraulis capensis*), are also commercially important. Accurate information about hake feeding and their interaction with other organisms in their environment is important for effective management. The use of stable isotope ratios as indicators of hake diet integrated over the turnover time of the tissues sampled will supplement the information derived from gut content analyses by indicating the diet of the hake over a longer time period than is possible by gut content analysis. Muscle tissue reflects the stable isotope ratios in the diet over a shorter time period than bone collagen (Sholto-Douglas 1991), thus diets over two different time periods are indicated. This may contribute to the effective management of the resource.

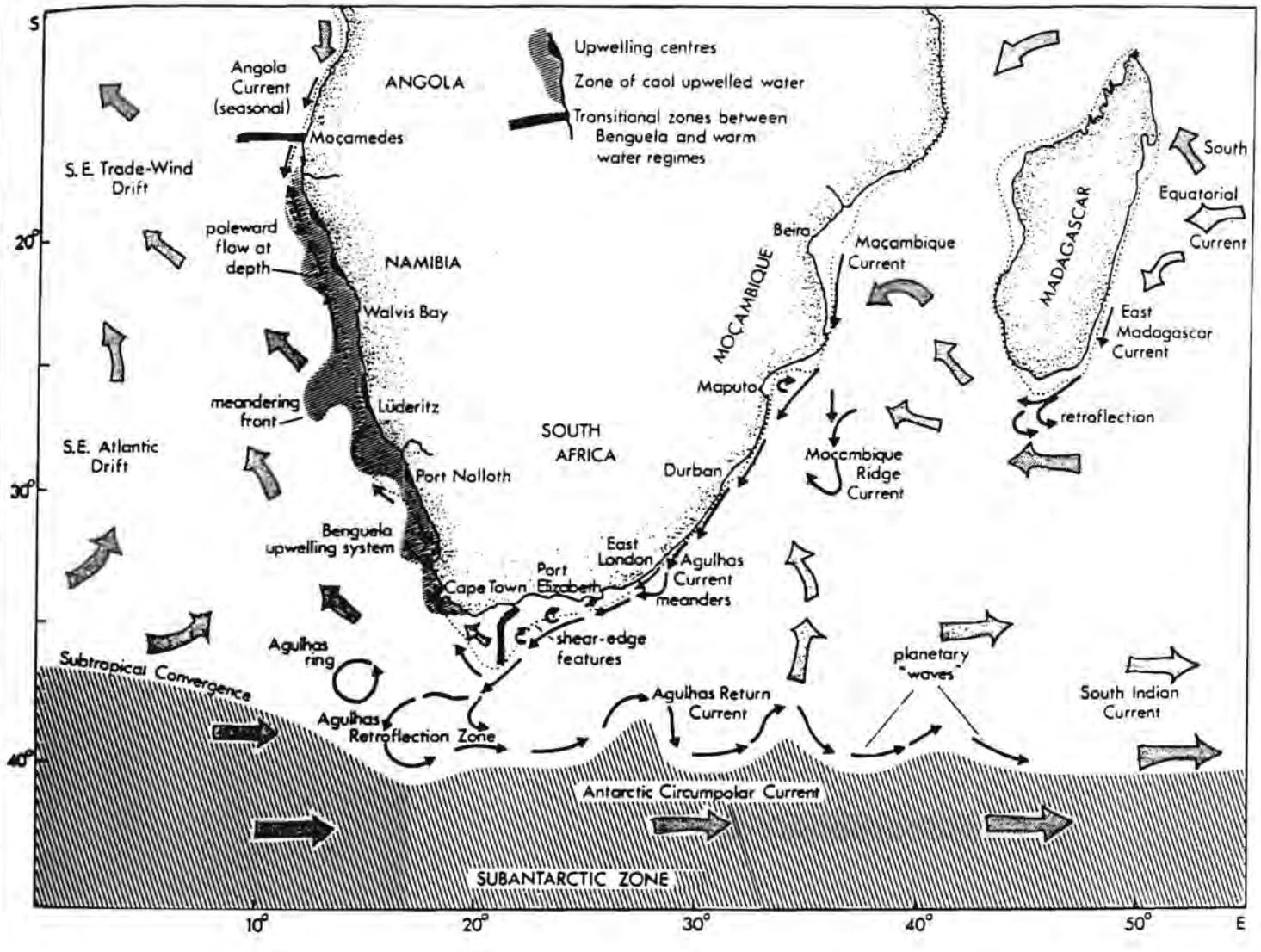
1.2. The Environment

The environments in which the hake are found are different on the south and west coasts. The surface waters of the coasts differ in physical, chemical and biological characteristics (Shannon 1989). This may affect the species composition of fish and plankton on the two coasts, as well as the isotopic composition of the hake diet and the hake themselves. A short overview highlighting the main differences between the Benguela system on the west coast and the south coast and Agulhas bank is therefore given below. Fig. 1.2.1 shows a summary of the physical oceanography around the South African coast.

1.2.1 The Benguela System

The Benguela system off the west coast of South Africa is very variable (Shannon 1989). The system is bordered by warm water regimes to the north, south, south-east and west, the boundaries being subject to fluctuations (op.cit.). The main characteristic of the Benguela system is wind-driven coastal upwelling (Barange *et al.* 1992) where longshore equatorial winds drive surface water offshore and cool, dense, nutrient-rich water upwells to take its place. This leads to high biological productivity in west coast waters (Shannon 1989). The upwelling is controlled mainly by bathymetry and the influence of orography on the wind field (Shannon 1985). The South Atlantic high pressure system, atmospheric pressure over the sub-continent and low-pressure systems which move in an easterly direction determine the prevailing winds on the west coast (Shannon 1989). Near the coast the wind is mainly southerly or alongshore as the anticlockwise airflow from the South Atlantic high is guided by the coast (op.cit.). There is a seasonal variation in the pressure gradient along the west coast, the interior pressure changing from a weak high in winter to a strong low in summer, being particularly evident in the south. The south-easter is therefore the prevailing wind in summer (op.cit.), providing favourable conditions for upwelling. Cool, dense, deep central water upwells from about 300m to replace surface water moved offshore and northwards by the south-east winds. The sea surface temperature is low, averaging between 13-15°C. The

Fig. 1.2.1



Diagrammatic summary of the physical oceanography around the southern African coast. The Benguela system on the west coast and the Agulhas current on the east and south coasts are indicated. (From Shannon 1989).

temperature varies with season and area (op.cit.). The intensity of upwelling is not uniform along the coast. Important upwelling centres are found off the Cape Peninsula, Cape Columbine and Hondeklip Bay (Taunton-Clark 1985 in Pagès *et al.* 1991) in areas where the continental shelf along the African coast is narrowest and the wind strongest (Shannon 1989). Thermoclines are uncommon in the Benguela system, and if present are weak, occurring in the shallow areas of the shelf. (op.cit.). Since upwelling brings water which is rich in nutrients to the surface, conditions are favourable for the formation of phytoplankton blooms (op.cit.). The nutrients are utilised by the phytoplankton and are taken into the food web as part of the diet of herbivores.

1.2.2. The south coast and Agulhas bank

The Agulhas bank and the south coast have not been studied as intensively as the Benguela system (Boyd *et al.* 1992). The oceanography on the south coast is dominated by th influence of the Agulhas current. The Agulhas current flows in a south-easterly direction along the South African coast until it reaches the Port Elizabeth area, where it moves away from the coast (see Fig.1.2.1.). The surface water of the current is comprised of warm water of low salinity from the Moçambique current, and higher salinity subtropical surface water (op.cit.). Deeper water originates from regions south and east of Madagascar. Surface current speeds of 2.6 $m.s^{-1}$ have been recorded, the average being 1-2 $m.s^{-1}$ (Shannon 1989).

The Agulhas bank is the area between the Agulhas current and the Benguela system, where the majority of south coast fish samples analysed in this study were caught (see Fig.1.2.1.). The oceanography in this area is complex, and not well understood. Locally important wind-induced upwelling does occur in this area close inshore, particularly near capes such as Recife, Agulhas, St Francis and St Blaize (op.cit.), but not nearly to the same extent as in the Benguela system. The sea-surface temperatures over the Agulhas bank range from 20-21°C on summer to 16-17°C in winter (op.cit.). Water may be of Atlantic or Agulhas current origin. In summer solar heating of the surface water, which is mixed to 30-40m, creates a thermocline. Westerly gales in winter cause extensive mixing of the water and insolation is decreased so that the

thermocline is not present, deeper water being colder in summer than in winter (Shannon 1989). Nutrient concentrations in the surface waters of the Agulhas bank are lower than off the west coast, but a relatively productive marine community is supported. Nutrient recycling is probably common (op.cit.).

1.3. Stable carbon and nitrogen isotope ratios

Stable carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios have been found to reflect the diet of a consumer (Miyake and Wada 1967, DeNiro and Epstein 1978, 1981, Sealy *et al.* 1987, Sholto-Douglas *et al.* 1991), the consumer having higher levels of ^{13}C and ^{15}N than its diet. Stable isotope ratios are expressed in delta (δ) notation and are calculated according to the following formula:

$\delta X\ (^{o}/_{oo}) = [(R_{sample} - R_{standard})/R_{standard}] * 1000$

where X = ^{13}C or ^{15}N, R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$, the standard for $\delta^{13}C$ being Peedee Belemnite carbonate (Craig 1957) and atmospheric air for $\delta^{15}N$ (Mariotti 1983, 1984).

Consumers are enriched by 1-2$^{o}/_{oo}$ in ^{13}C (DeNiro and Epstein 1978, Macko *et al.* 1982, Fry and Sherr 1984, Sholto-Douglas *et al.* 1991), and 3-5$^{o}/_{oo}$ in ^{15}N (DeNiro and Epstein 1981, Minagawa and Wada 1984, Fry 1988, Sholto-Douglas *et al.* 1991). Ecosystem energetics and trophic level enrichments are detectable in this manner, the trophic positions of organisms in the food web being indicated (Rau *et al.* 1983, 1989, 1990, 1992, Fry and Sherr 1989, Schell and Ziemann 1989, Hobson and Welch 1992). According to Macko *et al.* (1982), food chains cannot be reconstructed by the use of stable carbon isotopes alone as different food sources may be isotopically indistinguishable. The presence of multiple carbon sources may make interpretations of the origins of carbon pathways difficult (Dunton and Schell 1987). Stable nitrogen isotope ratios are therefore used with $\delta^{13}C$ in this study as a complementary source of information. Two tracers reduce the possibility of carbon and nitrogen sources being isotopically indistinguishable.

The isotopic composition of various tissues in an animal may differ. DeNiro and Epstein (1981) found that muscle tissue in *Mus musculus* was enriched in ^{15}N relative to bone collagen when the animals were raised on a diet of constant isotopic composition. This is possibly due to different rates and degrees of fractionation of carbon and nitrogen during assimilation into the different tissues (DeNiro and Epstein 1981, Tieszen *et al.* 1983), and the variations in amino acid composition of the tissues analysed (Hare *et al.* 1991). The $\delta^{13}C$ values of bone collagen and muscle in the same animal can differ by more than $2^{o}/_{oo}$ (Fry and Sherr 1984, Sholto-Douglas *et al.* 1991).

According to Tieszen *et al.* (1983), metabolically active tissues have faster turnover rates than those which are metabolically inactive. In metabolically active tissues, changes in diet would be reflected more rapidly in the isotopic composition than in metabolically slower tissues. The isotopic composition of carbon and nitrogen which is eaten by an animal must be equal to the isotopic composition of body carbon and nitrogen, and that which is lost by respiration or excretion (DeNiro and Epstein 1981). DeNiro and Epstein (1978) therefore recommend using whole animals for stable isotope analysis. In the case of large animals this is not practical. To obtain an accurate measurement of the isotopic composition of an animal, and therefore the integrated dietary carbon and nitrogen over a period of time, it is necessary to analyse more than one type of tissue (Tieszen *et al.* 1983). The isotopic composition of muscle reflects the recent diet of the animal, and that of bone collagen reflects the integration of isotopes over a longer period of time. The average long-term diet, immediate diet and dietary shifts are measured in this manner. In this study $\delta^{13}C$ and $\delta^{15}N$ of both the muscle tissue and bone collagen of hake are compared to the isotopic composition of the gut contents and potential prey.

The trophic position of hake on the west coast has been investigated by Payne *et al.* (1987). Intensive gut content analyses were used. It was concluded that hake are able to adapt to changes in prey availability (Payne *et al.* 1987). Variation in the diet occurred geographically and with prey availability. Gut content analyses are able to provide 'snapshot' views of the fish diet in the very recent past (Monteiro *et al.* 1991, Sholto-Douglas *et al.* 1991). Depending on

the method of gut content analysis used, feeding intensity and diel feeding patterns may be estimated (Hyslop 1980), and prey species identified. This may not be adequate to identify long-term dietary patterns. For this reason stable carbon and nitrogen isotope ratios as indicators of diet, used in conjunction with gut content analyses could provide and indication of hake diet integrated over the turnover time of the tissues studied.

Trophic level enrichment of $\delta^{13}C$ is approximately $1^{o}/_{oo}$ per trophic level and that of $\delta^{15}N$ is $3^{o}/_{oo}$ per trophic level. The main aim of this study is to use $\delta^{13}C$ and $\delta^{15}N$ to estimate the trophic position of *Merluccius capensis* off the South African coast. Other effects, such as isotopic variation with season, area, fish length and tissue type are also evident in the data. These results will be presented and discussed. All results contribute to the available knowledge of the diet and habits of *M.capensis*.

2. METHODS

2.1. Fish sampling

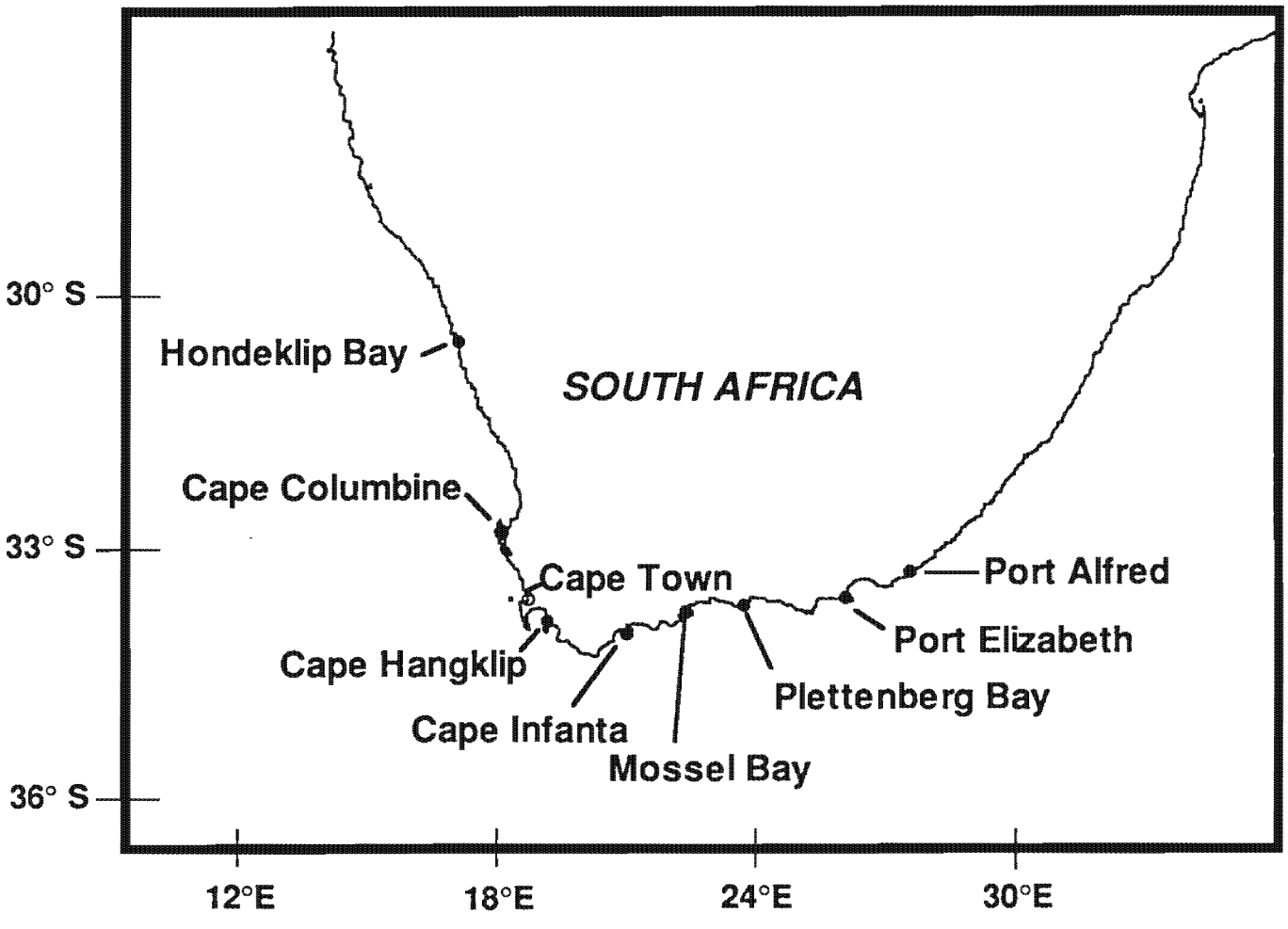
Merluccius capensis and prey fish samples were collected off the South African coast during hake biomass survey cruises and an anchovy recruitment survey cruise aboard the Sea Fisheries Research Institute vessel F.R.S. *Africana*. The fish were collected from the west coast in the Hondeklip Bay, Cape Columbine and Cape Hangklip areas (Fig. 2.1), Hondeklip Bay and Cape Columbine being upwelling centres (Crawford *et al.* 1987). Cape Infanta, Mossel Bay, Plettenberg Bay, Port Elizabeth and Port Alfred are the south coast areas in which samples were collected (Fig. 2.1). The west coast samples were collected during July 1990 (winter), January 1991 (summer), May 1991 and February 1992. Samples from the south coast were collected in June 1991.

Large hake were collected demersally during the day, as hake migrate upwards to feed in midwater at night (Payne *et al.* 1987). A German 180ft bottom trawl was used. Trawl duration was half an hour, and hake were collected from trawls 60-180m in depth. Some small fish, particularly on the south coast were taken from the shallower bottom trawls. The remainder of the small fish were collected on inshore cruises by midwater trawls on the west coast in July 1990 and May 1991. Midwater trawls were undertaken at night using an Engels 308 Midwater trawl.

The hake were divided into the following size classes (total length): ≤20cm, 21-40cm, 41-60cm, 61-80cm and >80cm.
At least five hake, one from each size class, from each area were analysed; 21 from the west coast and 32 from the south coast.

Potential hake prey fish present in each trawl were collected for isotopic comparison with hake tissue and gut contents. These include *Merluccius paradoxus* (deep-water hake), *Coelorhynchus fasciatus* (rattail), *Sardinops sagax ocellatus* (pilchard), *Engraulis capensis* (anchovy),

Fig. 2.1.



Areas in which hake samples were collected. Hondeklip Bay, Cape Columbine and Cape Hangklip are west coast sites. Cape Infanta, Mossel Bay, Plettenberg Bay, Port Elizabeth and Port Alfred are the south coast sites.

Cynoglossus zanzibarensis (sandrat), *Sepia australis, Paracallionymus costatus* (dragonet), *Maurolicus muelleri* (lightfish), *Trachurus trachurus capensis* (Cape horse mackerel), *Lampanyctodes hectoris* (lanternfish) and *Helicolenus dactylopterus* (jacopever).

Muscle and bone were removed from the dorso-anterior section of each hake. Gut contents were removed for analysis. Muscle of prey fish was used.

2.2. Zooplankton sampling

Samples of zooplankton were collected from the west coast during July 1990 and May 1991 during inshore cruises. The samples were collected on the same cruises as the small fish samples. The plankton was collected with a multiple opening/closing rectangular midwater trawl (RMT 1*6). Samples were divided into 200-500μm, 500-3500μm and >3500μm size classes. Whole individuals of zooplankton were used for analysis.

2.3. Sample Preparation

Lipids were removed from the fish and zooplankton samples by soaking in a solution of chloroform, methanol and distilled water (proportions 2:1:0.8) (Bligh and Dyer 1959). According to DeNiro and Epstein (1977) the $\delta^{13}C$ value of the lipid fraction of an organism is lower than the major protein and carbohydrate fraction, and 2-4$^{o}/_{oo}$ lower than that of the total organism. The basis for the lower values may be isotope effects during glucose metabolism (op.cit.). Sholto-Douglas (1992) shows that hake muscle and zooplankton samples containing lipids have more negative $\delta^{13}C$ values than those from which lipids have been extracted. Removal of lipids from samples may therefore have important implications in foodweb studies, particularly those concerning zooplankton.

The lipids eaten by a consumer are not broken down and resynthesised as carbohydrate or protein, but are either used as an energy source or are incorporated into the tissue of a consumer as lipids (Krueger and Sullivan 1984, Klepinger and Mintel 1986, Lee-Thorp *et al.*

1989, Ambrose and Norr 1993). If the lipids are removed from all the samples used this effect may not be detectable. However, as consumers will obviously ingest different quantities of lipids, isotopic and quantitative analysis of extracted lipids should be undertaken. Bone collagen will reflect the $\delta^{13}C$ of the protein of the prey, while carbon in the mineral portion of the bone preferentially reflects the energy portion of the diet (Krueger and Sullivan 1984). In this study only the collagen portion of bone is used, bone collagen preferentially assimilating dietary protein with little energy contributions (Ambrose and Norr 1993). It has not been determined whether lipid extraction has any effect on the $\delta^{13}C$ values of bone collagen in hake.

I decided to remove the lipids from the samples in this study to minimise isotopic discrepancies caused by different lipid proportions in the samples. Dietary protein is preferentially assimilated in hake tissue, particularly in bone collagen (Ambrose and Norr 1993). Removing the lipids from the samples would therefore not affect the stable isotope ratios in the tissue protein which reflect $\delta^{13}C$ and $\delta^{15}N$ in dietary protein. Furthermore, extraction of lipids from the hake samples will allow the stable isotope ratios obtained in this study to be compared to those in pelagic fish from the same area studied by Sholto-Douglas (1992), as these fish form part of the hake diet.

After lipid extraction the muscle, gut content and plankton samples were freeze-dried for 24 hours. Cleaned vertebral bone samples were decalcified in a 1.0M hydrochloric acid solution for 12 hours and then rinsed in distilled water to a neutral pH. They were defatted again and the remaining collagen freeze-dried, following standard methods reported by Sealy *et al.* (1987) and Sholto-Douglas *et al.* (1991).

2.4. Laboratory Isotope Analysis

Eight to 15mg of sample were weighed into quartz breakseal tubes with silver foil, excess copper metal and copper oxide. The tubes were evacuated to 10^{-2} Torr and sealed by flaming. Samples were combusted at 800°C for 6 hours and allowed to cool for 12 hours. CO_2 and N_2

were collected by cryogenic distillation on a vacuum line (Handley *et al.* 1991). N^2 was collected on coconut charcoal in a quartz breakseal tube at the temperature of liquid nitrogen (Sealy *et al.* 1987). Carbon dioxide was collected in a pyrex tube by freezing with a dry ice and ethanol mixture. The isotope ratios of the separate gases were measured using a VG Micromass 602E 90° double collector mass spectrometer.

Natural isotopic differences are significant, but small when measured in atomic percent (Krueger 1984, Krueger and Sullivan 1984). Isotopic results are therefore expressed in delta (δ) notation to amplify the differences between the natural stable isotopes of an element.

The following equation is used:

$$\delta X = [(R_{sample} - R_{standard})/ R_{standard}] * 1000\ ^{o}/_{oo}$$

where $X = \delta^{13}C$ or $\delta^{15}N$

$R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$

The standard for $\delta^{13}C$ is PDB (PeeDee Belemnite carbonate) (Craig 1957, DeNiro and Schoeninger 1983) and that for $\delta^{15}N$ is atmospheric nitrogen (Mariotti 1983, 1984, Mizutani *et al.* 1991, Sholto-Douglas 1992). The $\delta^{13}C$ and $\delta^{15}N$ values were calibrated using Merck gelatine (M1360, UCT# 1886) according to the PDB (Craig 1957) and atmospheric nitrogen (Mariotti 1983) standards respectively.

2.5. Statistics

Lord's Range test was used to compare small samples ($n < 5$), and the data were calculated according to Langley (1968). Multivariate and one-way analysis of variance tests for comparison of larger sample sizes were calculated according to Zar (1984). Power curves were fitted to certain data, and Spearman's rank correlation calculated according to Zar (1984).

3. RESULTS

3.1 Seasonal differences in stable isotope ratios in hake

The seasonal differences in $\delta^{13}C$ and $\delta^{15}N$ were measured from fish muscle, bone collagen and gut content samples obtained from Cape Columbine during July 1990 and February 1992. The fish were divided into two size classes; $\geq$40cm and $<$40cm as, according to Botha (1985), hake over 40cm in length are regarded as mature. The diet of hake smaller than 40cm is dominated by crustaceans (Ware 1992). The data for the $\delta^{13}C$ values are shown in Table 3.1.1 and those for $\delta^{15}N$ in Table 3.1.2. The sample size (n) for seasonal comparison is small. Lord's Range Test (Langley 1968) was used to detect differences between the small sample means as it is particularly suited to small sample sizes.

$\delta^{13}C$ in July was found to be significantly lower than in February in the muscle tissue ($p<0.01$) and bone collagen ($p<0.01$) of small hake ($<$40cm) (Table 3.1.3). No significant seasonal differences in $\delta^{13}C$ were found in the gut contents of the small fish and the muscle, bone collagen and gut contents of the large fish ($\geq$40cm), although $\delta^{13}C$ in the bone collagen of the large hake seems to be slightly more positive in winter. It appears that the $\delta^{13}C$ of muscle and bone collagen in the small fish represents an integration of dietary $\delta^{13}C$ over a period of six months or less, while the integration of dietary $\delta^{13}C$ in large fish, to a stage where it is isotopically detectable in muscle and bone collagen, takes longer than the six month period studied. This could be due to either a slower metabolic incorporation of prey carbon into the tissues of larger fish, or a diet that does not change seasonally. There is no significant difference in the $\delta^{13}C$ of the gut contents between the February and July samples from small fish. As the long-term dietary picture appears to be different, this may be due to fish feeding on atypical prey items at the time caught.

No significant seasonal differences in $\delta^{15}N$ were found in the muscle, bone collagen or gut contents of small or large hake (Table 3.1.3). $\delta^{13}C$ may therefore be a better indicator of seasonal changes in diet than $\delta^{15}N$, unless the diet of hake changes to incorporate seasonal

TABLE 3.1.1.

FISH LENGTH/ SEASON	MUSCLE	BONE COLLAGEN	GUT CONTENTS	n
≥40cm February	-15.58 (0.60)	-15.0 (0.92)	-16.02 (0.66)	4 (S.D.)
≥40 cm July	-14.67 (0.54)	-13.5 (0.59)	-15.08 (0.44)	3 (S.D)
<40cm February	-16.15 (0.21)	-16.62 (0.02)	-17.79 (0.03)	2 (S.D.)
<40cm July	-15.53 (0.14)	-13.07 (1.10)	-16.38 (1.65)	3 (S.D.)

$\delta^{13}C$ in two sizes of hake during February and July at Cape Columbine. The standard deviation of the sample mean is indicated in brackets below the mean value.

TABLE 3.1.2.

FISH LENGTH/ SEASON	MUSCLE	BONE COLLAGEN	GUT CONTENTS	n
≥40cm February	16.17 (0.74)	15.16 (0.35)	13.94 (0.82)	4 (S.D.)
≥40cm July	16.56 (0.83)	15.44 (1.11)	15.07 (0.89)	3 (S.D.)
<40cm February	13.5 (0.76)	12.86 (0.09)	12.45 (1.11)	2 (S.D.)
<40cm July	14.99 (0.59)	12.69 (1.39)	13.08 (2.33)	3 (S.D.)

$\delta^{15}N$ in two size classes of hake during February and July at Cape Columbine. The standard deviation of the sample mean is indicated in brackets under the mean value.

TABLE 3.1.3.

CAPE HAKE (LENGTH ≥40cm)						
	MUSCLE		BONE COLLAGEN		GUT CONTENTS	
	L	P	L	P	L	P
$\delta^{13}C$	0.38	n.s.	0.48	n.s.	0.43	n.s.
$\delta^{15}N$	0.12	n.s.	0.10	n.s.	0.36	n.s.
CAPE HAKE (LENGTH <40cm)						
	MUSCLE		BONE COLLAGEN		GUT CONTENTS	
	L	P	L	P	L	P
$\delta^{13}C$	2.80	$p<0.01$	8.59	$p<0.01$	0.75	n.s.
$\delta^{15}N$	0.78	n.s.	0.09	n.s.	0.13	n.s.

Statistics undertaken to determine whether $\delta^{13}C$ and $\delta^{15}N$ manifest seasonal changes in the muscle, bone collagen and gut contents of *Merluccius capensis*. L is the test statistic for Lord's Range Test (Langley 1968). P is the probability that the tissue tested has no seasonal change in stable isotope ratios (n.s. = no significant seasonal difference).

Table 3.1.4.

JULY		
Size class (μm)	$\delta^{13}C$	$\delta^{15}N$
200-500	-18.17	7.19
500-3500	-18.76	8.03
>3500	-17.03	9.5
FEBRUARY		
200-500	-16.09	9.34
500-3500	-16.10	8.44
>3500	-17.09	9.57

$\delta^{13}C$ and $\delta^{15}N$ in different size classes of zooplankton sampled from the Cape Columbine area during July 1990 and February 1992. It was only possible to collect enough material for one sample in each zooplankton size class.

food sources with very different proportions of nitrogen isotopes. This does not appear to occur with large or small hake on the west coast of South Africa. Unfortunately no data were available for seasonal comparison of hake isotope ratios on the south coast. Isotopic enrichment between a consumer and its diet is 1-2$^{o}/_{oo}$ for $\delta^{13}C$ and 3-4$^{o}/_{oo}$ for $\delta^{15}N$ (Fry and Sherr 1984, Minagawa and Wada 1984, Fry 1988). Seasonal differences in $\delta^{15}N$ on the west coast may be too small to be statistically significant, or too much variation may be present in the small samples to detect differences.

Table 3.1.4 shows the $\delta^{13}C$ and $\delta^{15}N$ values in zooplankton sampled at the same time as the fish. Lord's range test carried out on the data indicates that there is a significant difference in $\delta^{13}C$ between February and July in the pooled 200-500μm and 500-3500μm size classes ($L=3.95$, $p<0.05$). There was no significant difference in $\delta^{15}N$ with season. This could be due to the effect of zooplankton size classes on the data as too few data were available to analyse each size class separately. No isotopic variation with season was found in the >3500 size class.

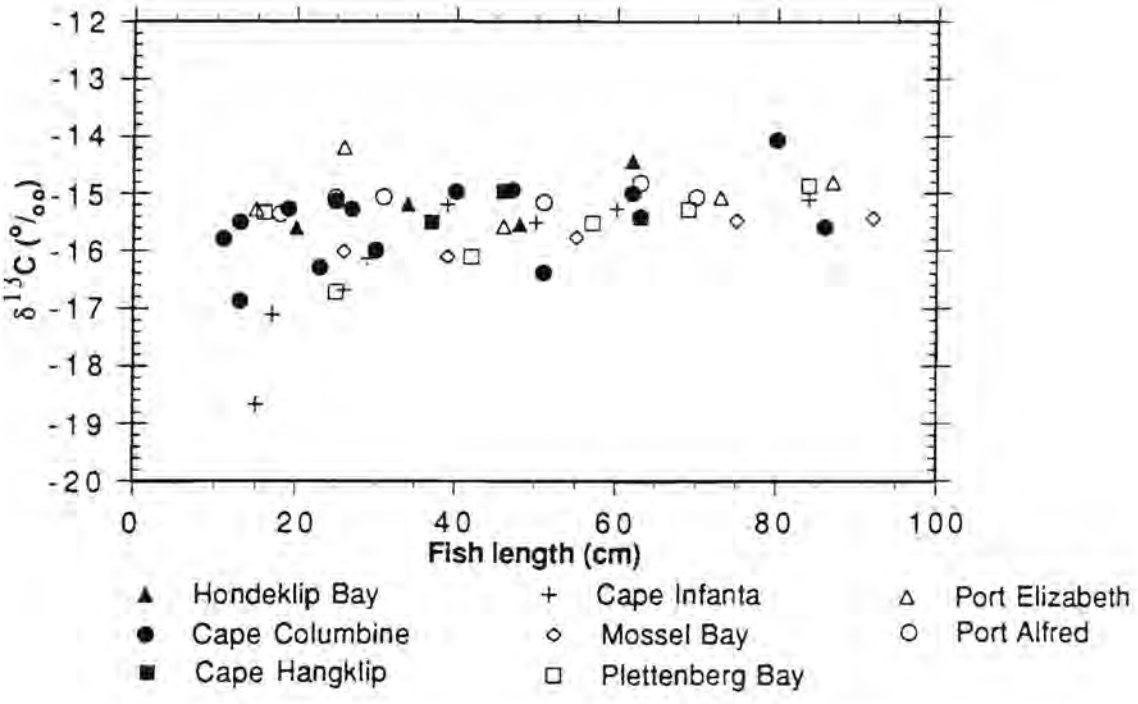
A larger sample size and more frequent sampling are necessary in order to draw feasible conclusions about the seasonal differences in hake tissues and zooplankton. This was not possible in this study as sampling was dependant on scheduled cruises. Conclusions drawn about seasonal differences in the present data are preliminary.

3.2 Geographical differences in stable isotope ratios

Samples were obtained from three areas on the west coast of South Africa: Hondeklip Bay, Cape Columbine and Cape Hangklip; and five areas on the south coast: Cape Infanta, Mossel Bay, Plettenberg Bay, Port Elizabeth and Port Alfred.

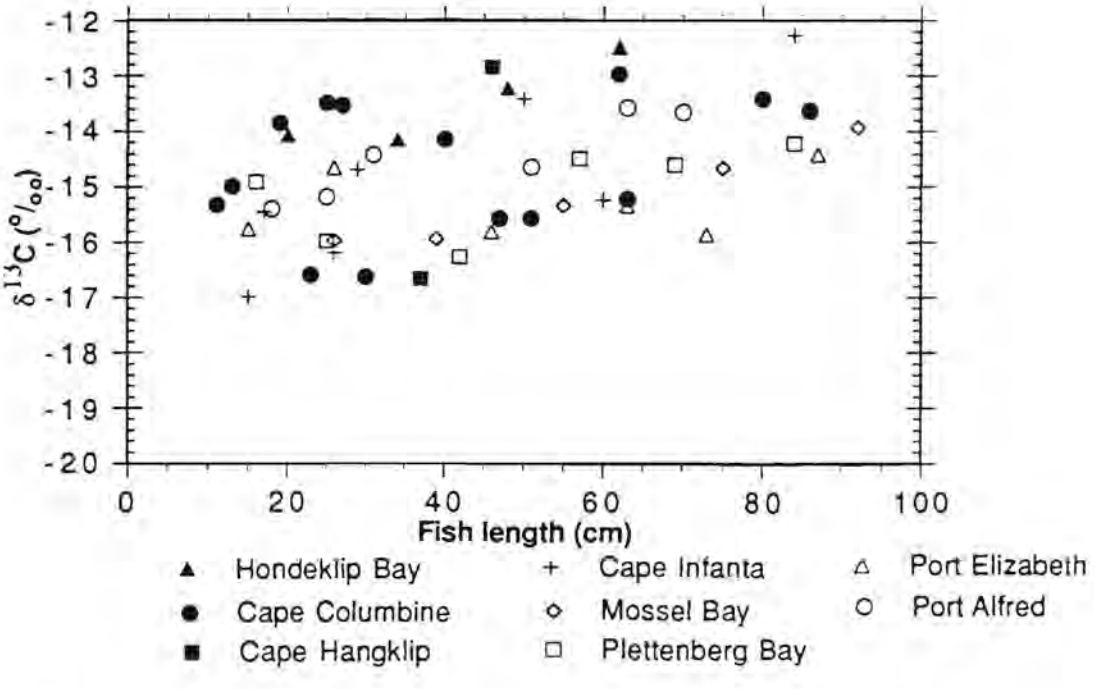
Fig. 3.2.1 shows the $\delta^{13}C$ values of hake muscle on the south and west coasts in the different areas. A two-way analysis of variance was performed on the data set to find whether the stable isotope ratios varied with fish length and area. There is no significant difference in the $\delta^{13}C$ in

Fig. 3.2.1.



$\delta^{13}C$ in the muscle of different size hake from all sample areas. Solid symbols represent west coast sample areas, and open symbols south coast areas.

Fig. 3.2.2.



$\delta^{13}C$ in the bone collagen of different size hake from all sample areas. Solid symbols represent west coast sample areas, and open symbols south coast areas.

TABLE 3.2.1.

MUSCLE					
Source of variation	**Sum of squares**	**d.f.**	**Mean square**	**F**	**Sig. level**
Area	0.59	1	0.59	1.14	0.29
Length	7.52	4	1.88	3.64	**0.01**
A vs L	0.26	4	0.06	0.12	0.97
Residual	21.70	42	0.52		
Total	29.77	51			
BONE COLLAGEN					
Area	7.71	1	7.71	6.01	**0.02**
Length	13.27	4	3.32	2.59	0.05
A vs L	2.95	4	0.74	0.58	0.68
Residual	52.54	41	1.28		
Total	74.44	50			
GUT CONTENTS					
Area	3.98	1	3.98	2.88	0.10
Length	4.83	4	1.21	0.87	0.49
A vs L	4.81	4	1.20	0.87	0.49
Residual	56.60	41	1.38		
Total	224.40	49			

Results of a two-way ANOVA done on $\delta^{13}C$ values of fish from different length classes and areas. F is the test statistic for the analysis of variance. The numbers in bold text indicate a significant difference in $\delta^{13}C$ with area or fish length. D.f. signifies degrees of freedom.

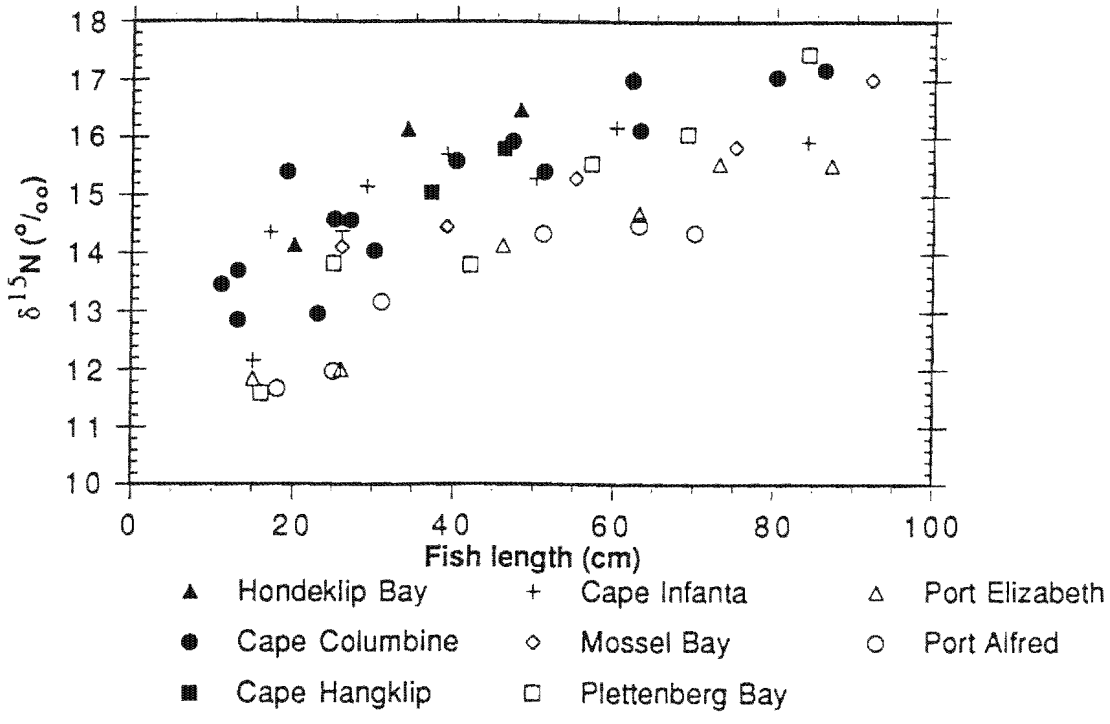
hake muscle between the west and south coasts (p=0.2928) (Table 3.2.1). However $\delta^{13}C$ is significantly higher in hake bone on the west coast than on the south coast (Fig.3.2.2, Table 3.2.1). Muscle tissue integrates dietary carbon faster than bone collagen and therefore reflects the diet of an organism over a shorter time period than bone collagen. As $\delta^{13}C$ in the muscle of hake probably reflects the diet over a shorter period of time than $\delta^{13}C$ in hake bone collagen, this may indicate a short term diet change on either the west or the south coast. The exact turnover time of hake muscle tissue and bone collagen is not known. No interaction was found between fish length and area.

The $\delta^{15}N$ values for hake muscle and bone in the different areas are shown in Fig.3.2.3 and Fig.3.2.4 respectively. The results of the two-way ANOVA performed on these data are shown in Table 3.2.2. $\delta^{15}N$ is significantly higher in hake muscle on the west coast than on the south coast (p=0.0002), as well as in hake bone collagen (p=0.0001). The $\delta^{15}N$ values appear to be particularly low in the Plettenberg Bay area and decline further at Port Elizabeth and Port Alfred. This trend is evident in the $\delta^{15}N$ of bone collagen. No interaction was found between fish length and area (Table 3.2.1 and Table 3.2.2).

The $\delta^{13}C$ and $\delta^{15}N$ values of the hake gut contents were measured. There was no significant difference in $\delta^{13}C$ with area (p=0.097). $\delta^{15}N$ of the gut contents was significantly higher on the west coast (p=0.0005).

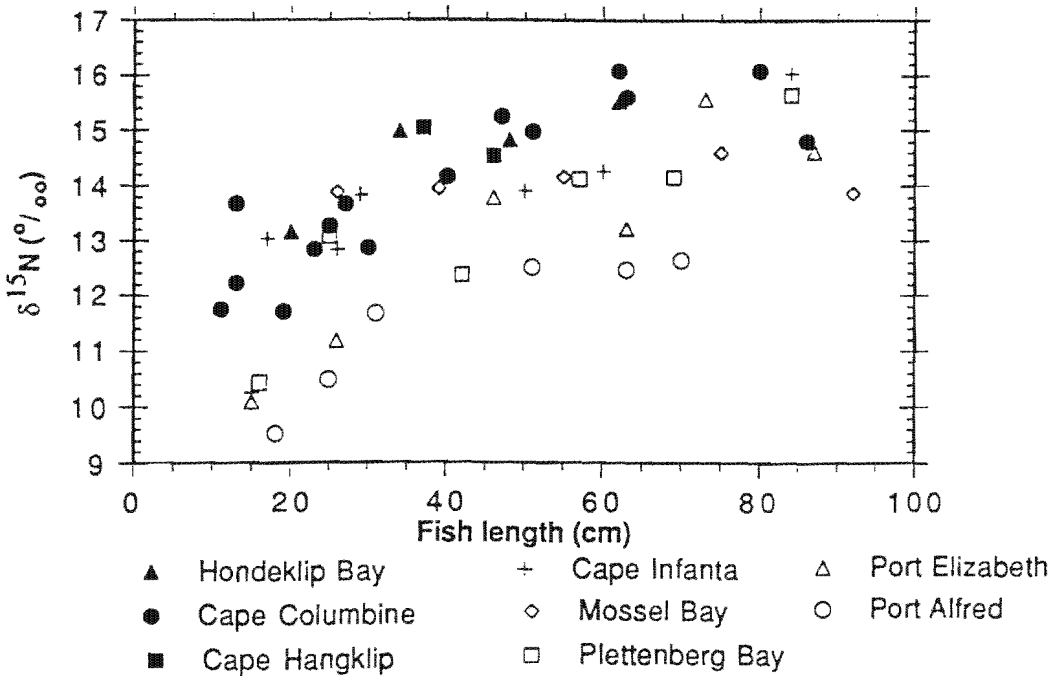
If the isotopic differences observed on both coasts in $\delta^{15}N$ in muscle and bone collagen are dietary, they may be traceable to the primary production of the relevant food web. The higher $\delta^{15}N$ on the west coast may be caused by variation in the availability of different sources of inorganic nitrogen (ammonium and nitrate), which have different values of $\delta^{15}N$, between the coasts, or different species composition of phytoplankton and/or zooplankton leading to more trophic levels on the west coast.

Fig. 3.2.3.



$\delta^{15}N$ in the muscle of different size hake from all sample areas. Solid symbols represent west coast sample areas, and open symbols south coast areas.

Fig. 3.2.4



$\delta^{15}N$ in the bone collagen of different size hake from all sample areas. Solid symbols represent west coast sample areas, and open symbols south coast areas.

TABLE 3.2.2.

MUSCLE					
Source of variation	**Sum of squares**	**d.f.**	**Mean square**	**F**	**Sig. level**
Area	14.85	1	14.85	16.35	**0.00**
Length	74.03	4	18.51	20.38	**0.00**
A vs L	0.78	4	0.19	0.21	0.93
Residual	38.15	42	0.91		
Total	121.25	51			
BONE COLLAGEN					
Area	21.61	1	21.61	19.37	**0.00**
Length	71.63	4	17.91	16.05	**0.00**
A vs L	3.64	4	0.91	0.82	0.52
Residual	45.75	41	1.12		
Total	134.05	50			
GUT CONTENTS					
Area	38.15	1	38.15	14.11	**0.00**
Length	79.19	4	19.80	7.32	**0.00**
A vs L	7.41	4	1.86	0.69	0.61
Residual	108.12	40	2.70		
Total	224.39	49			

Results of a two-way ANOVA done on $\delta^{15}N$ values of fish from different length classes and areas. F is the test statistic for the analysis of variance. D.f. indicates degrees of freedom. The numbers in bold text indicate a significant difference in $\delta^{15}N$ with area or fish length.

3.3 Variation of isotope ratios with fish length and tissue type

Figs 3.2.1 to 3.2.4 show the variation of $\delta^{13}C$ and $\delta^{15}N$ in hake muscle and bone collagen with fish length as well as area. $\delta^{13}C$ increases slightly with increasing fish length in hake bone collagen, but this was not statistically significant. No curve could be fitted to the data on these graphs and Spearman's rank correlation was not significant.

The $\delta^{15}N$ values in Fig. 3.2.3 and Fig. 3.2.4 for hake muscle and hake bone collagen respectively show an increase in $\delta^{15}N$ with increasing fish length. Power curves were fitted to the data shown on these graphs. The power curve equations are:

West coast hake muscle: $N = 9.4831 * L^{0.13387}$ ($r^2=0.79$)

West coast hake bone collagen: $N = 8.5431 * L^{0.14172}$ ($r^2=0.76$)

South coast hake muscle: $N = 7.8648\ L^{0.16232}$ ($r^2=0.69$)

South coast hake bone collagen: $N = 6.5895\ L^{0.18223}$ ($r^2=0.60$).

Where L = Fish length (cm), N = $\delta^{15}N$ ($^{o}/_{oo}$).

The increase in $\delta^{15}N$ with fish length is initially rapid, but decreases as fish length increases. This could be due to a slower rate of incorporation of the dietary isotopes into the muscle and bone collagen of larger fish.

$\delta^{15}N$ in hake muscle is 1-1.5 $^{o}/_{oo}$ higher than that of hake bone collagen. A one-way ANOVA of the difference in $\delta^{13}C$ and $\delta^{15}N$ in muscle and bone collagen indicates that the muscle and bone collagen differ significantly in $\delta^{15}N$ ($F=7.855$, $p=0.0282$), but do not differ in $\delta^{13}C$ ($F=1.219$, $p=0.4809$). This could be due to the different amino acid composition of muscle and bone.

4. DISCUSSION

4.1 Seasonal differences in isotope ratios

Carbon

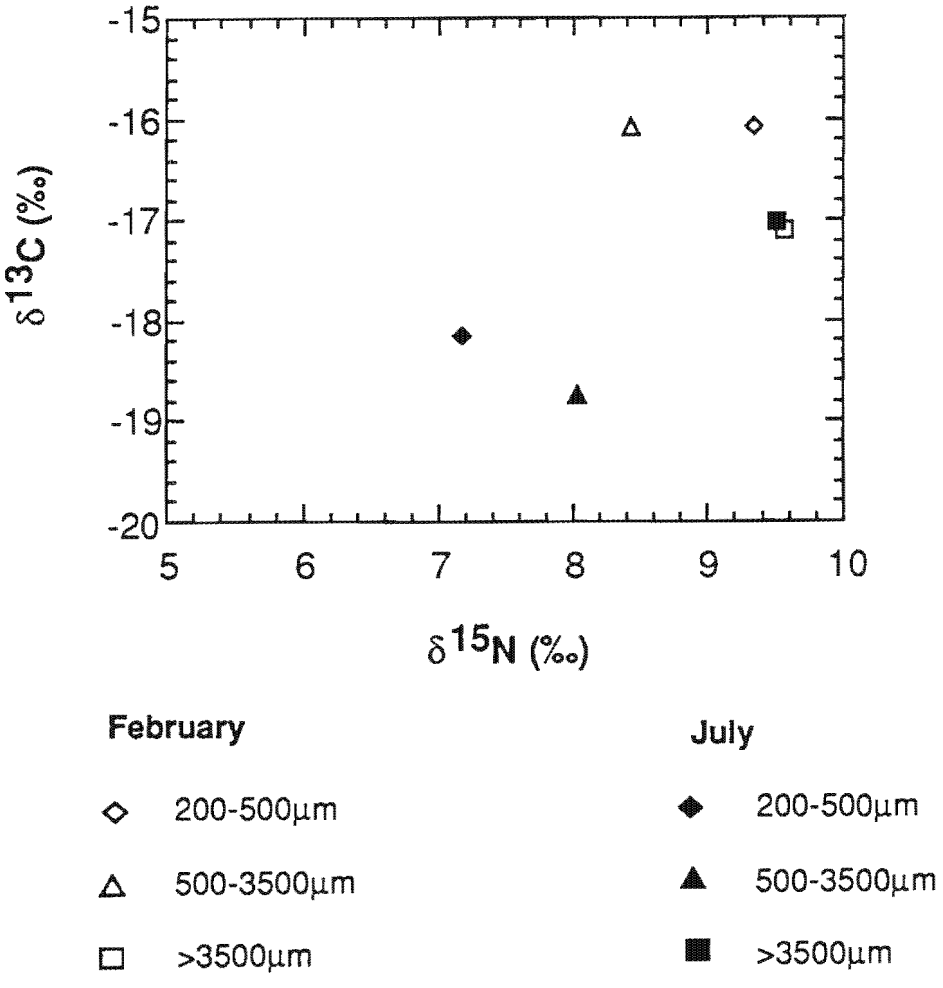
Zooplankton

The basis for the seasonal difference in $\delta^{13}C$ in small fish may be the evidence of seasonal differences in the isotopic composition of the carbon in their diet. There is no significant difference in the $\delta^{13}C$ values of the gut contents of the small fish with season. As the gut contents reflect only the immediate diet at the time of capture, it is not impossible that the fish were feeding on atypical prey at the time they were caught. The diet of the small hake may be isotopically more variable than is reflected in the muscle and bone collagen because the diet isotope ratios would be integrated over the time taken to incorporate them into the tissues.

Small hake are reported to feed mainly on crustaceans, although myctophid fish form a substantial part of the hake diet in the Cape Columbine area (Payne *et al.* 1987). 90% of the net plankton on the west coast is comprised of copepods (retained by 200μm mesh when sampled) and euphausiids (retained by 1600μm mesh) (Hutchings *et al.* 1991). Fig. 4.1.1 shows the seasonal difference in $\delta^{13}C$ versus $\delta^{15}N$ in different size classes of zooplankton. The 200-500μm size class (probably mainly copepods) and the 500-3500μm size class (probably mainly euphausiids) show higher $\delta^{13}C$ values in summer than in winter. No seasonal fluctuations in the standing stock of euphausiids occur on the west coast (Hutchings *et al.* 1991), but copepod stocks exhibit a summer maximum and a winter minimum (Pillar 1986). Copepods are mainly herbivorous, while euphausiids are omnivorous, 15-60% of ingested carbon being of phytoplanktonic origin, the remainder being made up by the consumption of copepods (Hutchings *et al.* 1991). The $\delta^{13}C$ of copepods would therefore influence the euphausiid isotopic composition, depending on the proportion of copepod carbon in the diet.

Cifuentes *et al.* (1988) found suspended particulate matter to be enriched in $\delta^{13}C$ in summer in the Delaware estuary. The higher $\delta^{13}C$ values were a result of faster primary productivity, associated with algal growth. These variations are not attributed to temperature effects, which

Fig. 4.1.1.



$\delta^{13}C$ versus $\delta^{15}N$ in various size classes of zooplankton sampled in February 1992 and July 1990 at Cape Columbine on the west coast. Isotope ratio values in the two smaller phytoplankton size classes appear to be higher in summer than in winter.

may be confused with isotopic changes caused by varying species composition (Cifuentes *et al.* 1988). Kalish (1991) found no relationship between temperature and $\delta^{13}C$ in the otoliths of Australian salmon (*Arripis trutta*). Simenstad and Wissmar (1985) report that $\delta^{13}C$ variation with season in estuarine and nearshore marine environments was common in primary producers, $^{13}C/^{12}C$ ratios being higher in summer. Effects of phytoplankton metabolism on carbon fractionation must be taken into account, and a thermal effect alone cannot be used to explain carbon isotopic variations (Descolas-Gros and Fontugne 1990).

Phytoplankton blooms on the west coast of South Africa develop in response to upwelling, when nutrient-rich water is introduced into the euphotic zone (Probyn 1985, Brown and Hutchings 1987). Upwelling is seasonal, occurring from September to April (Brown and Hutchings 1987). Diatoms are dominant in the phytoplankton on the west coast (Shannon and Pillar 1986, Brown and Hutchings 1987). Spring and summer blooms of diatoms on Georges bank are rich in $\delta^{13}C$ (-19 - -15$^{o}/_{oo}$), while the $\delta^{13}C$ of other phytoplankton and particulate organic matter is depleted (-25 - -21$^{o}/_{oo}$) (Fry and Wainright 1991). Diatom samples from the Atlantic and Pacific oceans and the Gulf of Mexico also show $\delta^{13}C$ enrichment (Fry and Wainright 1991). It would appear that the $\delta^{13}C$ enrichment in summer in the 200-500μm zooplankton size class is the result of seasonal variation in the abundance and species composition of diatom blooms forming in response to upwelling. Gearing *et al.* (1984) found seasonal differences in $\delta^{13}C$ between samples where diatoms were most abundant (winter/spring), and where nanoplankton dominated.

Sholto-Douglas *et al.* (1991) report enrichment in $\delta^{13}C$ with increasing plankton size, suggesting that larger plankton may feed further up the food web than do smaller plankton. There does not appear to be a large difference in $\delta^{13}C$ between the 200-500μm size class and the 500-3500μm size class in the seasonal data (Fig 4.1.1). Owing to the small sample size, no statistical analyses were undertaken to determine isotopic differences with season within size classes. However the 500-3500μm size class also shows ^{13}C enrichment in summer. This could be due to direct feeding on ^{13}C enriched diatoms, or to feeding on zooplankton which

had eaten the diatoms. The proportions of phytoplankton or small zooplankton consumed would therefore determine the extent of $\delta^{13}C$ fractionation reflected in the larger plankton.

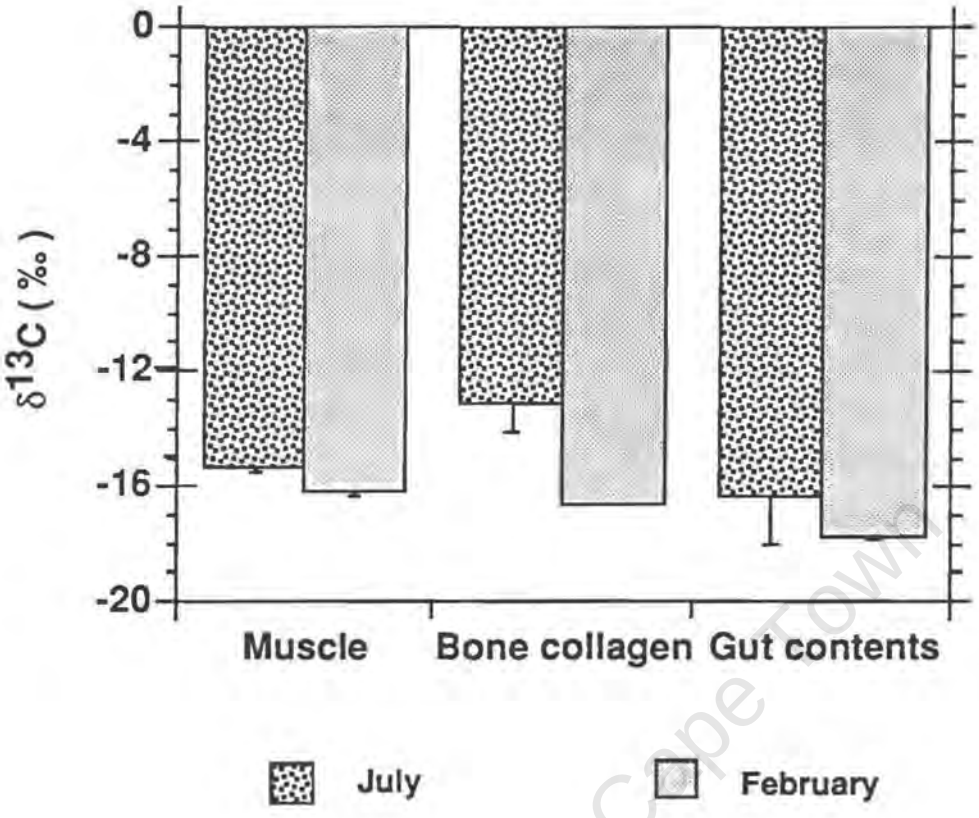
$\delta^{13}C$ values for the >3500μm size class appear to be very similar in February and July (Fig.4.1.1). However since this observation is based on two data points on which no statistical tests can be carried out, no conclusions can be drawn about $\delta^{13}C$ variation with season in this size class. However as the smaller zooplankton size classes show seasonal trends, it might be expected that these would be evident in the larger zooplankton if they feed one or more steps down the food web.

No effect of $\delta^{13}C$ variation in lipid proportion with season or geographical location could be examined in this study as the lipids were extracted from the samples. No isotopic analysis was done on the extracted lipids.

Small fish (<40cm)

There is a seasonal difference in $\delta^{13}C$ in the muscle and bone of small hake caught in the Cape Columbine area (Fig.4.1.2). The fish caught in February had significantly lower $\delta^{13}C$ values than those caught in July (see section 3.1). This indicates that the turnover time of the tissues sampled may be less than six months. $\delta^{13}C$ in muscle and bone of adult pilchard (*Sardinops sagax ocellatus*) did not change during a 35 week experiment when the diet was changed from trout pellets to snoek roe in the 13^{th} week (Sholto-Douglas 1992). The half-life of $\delta^{13}C$ in different tissues of gerbils was determined by Tieszen *et al.* (1983) after changing the diet of the animals. The half-life of $\delta^{13}C$ in the gerbil muscle was 27.6 days. Tissues such as fat and liver which have fast metabolic rates have faster turnover rates than tissues that are less metabolically active (Tieszen *et al.* 1983). Large animals have slower metabolic rates than small animals, and homeotherms have faster metabolic rates than poikilotherms (Schmidt-Nielsen 1984). The average standard metabolic rate of 57 fish incorporating 34 species from

Fig. 4.1.2.



$\delta^{13}C$ in the muscle, bone collagen and gut contents of small hake (<40cm) sampled in July 1990 and February 1992. Bars on histograms indicate standard deviation.

temperate waters is $0.089 mgO_2.g^{-1}.h^{-1}$ (Cowey and Sargent 1979). The basal metabolic rate of the gerbil *Gerbillus pusillus* is $1.05 mgO_2.g^{-1}.h^{-1}$ (Buffenstein 1984). It is evident that the metabolic rate of the gerbil is much faster than that of the fish tested, and therefore likely to be higher than that of hake. The turnover time of the muscle tissue of hake is therefore expected to be substantially longer than 27.6 days. It would appear that the turnover time in muscle and bone of hake less than 40cm in length is less than six months. The exact turnover rate of muscle tissue and bone collagen in hake is not known.

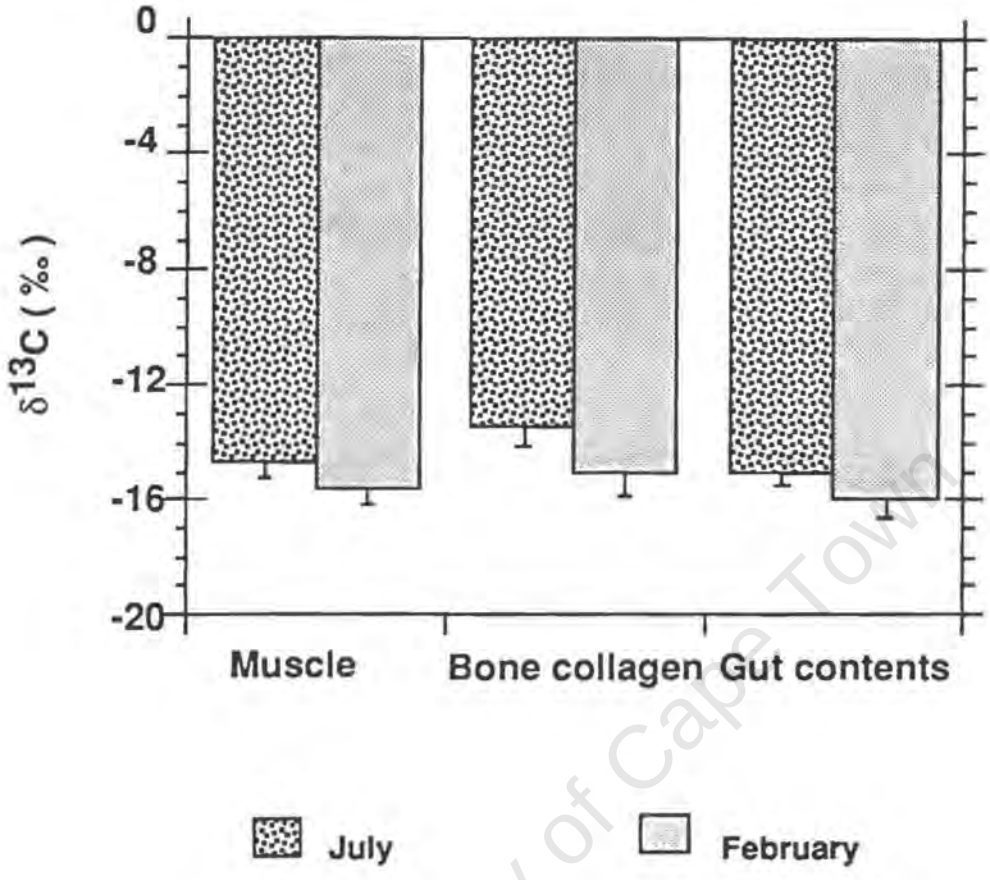
In Fig. 4.1.2. it appears that $\delta^{13}C$ in the gut contents of hake is more negative in winter (July) than in summer (February), as occurs with $\delta^{13}C$ in the zooplankton (see section 4.1.1.). This is the opposite to the carbon isotope values found in the muscle tissue and bone collagen of small hake. Cifuentes *et al.* (1988) found $\delta^{13}C$ to be higher in suspended particles in summer, when productivity was highest. Productivity is highest on the west coast in summer as this is the period when upwelling occurs (Brown and Hutchings 1987), thus the higher $\delta^{13}C$ values in the tissues of small hake in winter may be due to the time taken to incorporate the dietary carbon into the tissue.

Large fish (≥ 40cm)

Seasonal $\delta^{13}C$ values for the muscle, bone and gut contents of the large fish are shown in Fig.4.1.3. There is no statistically significant difference in the $^{13}C/^{12}C$ ratios with season. It was concluded that the turnover time of the tissues of small hake may be sufficiently fast to show the isotopic effects of changes in dietary $\delta^{13}C$ over a six month period. There are therefore two possible explanations for the lack of seasonal isotopic variations in large fish.

Firstly, the diet of the large fish may not vary with season. Hake are opportunistic feeders and eat whatever prey happens to be most abundant at the time (Payne *et al.* 1987). Roel and MacPherson (1988) studying the diet of *M.capensis* off the Namibian coast noted seasonal

Fig. 4.1.3



$\delta^{13}C$ in the muscle, bone collagen and gut contents of large hake (≥ 40cm) sampled in July 1990 and February 1992. Bars on histograms indicate standard deviation.

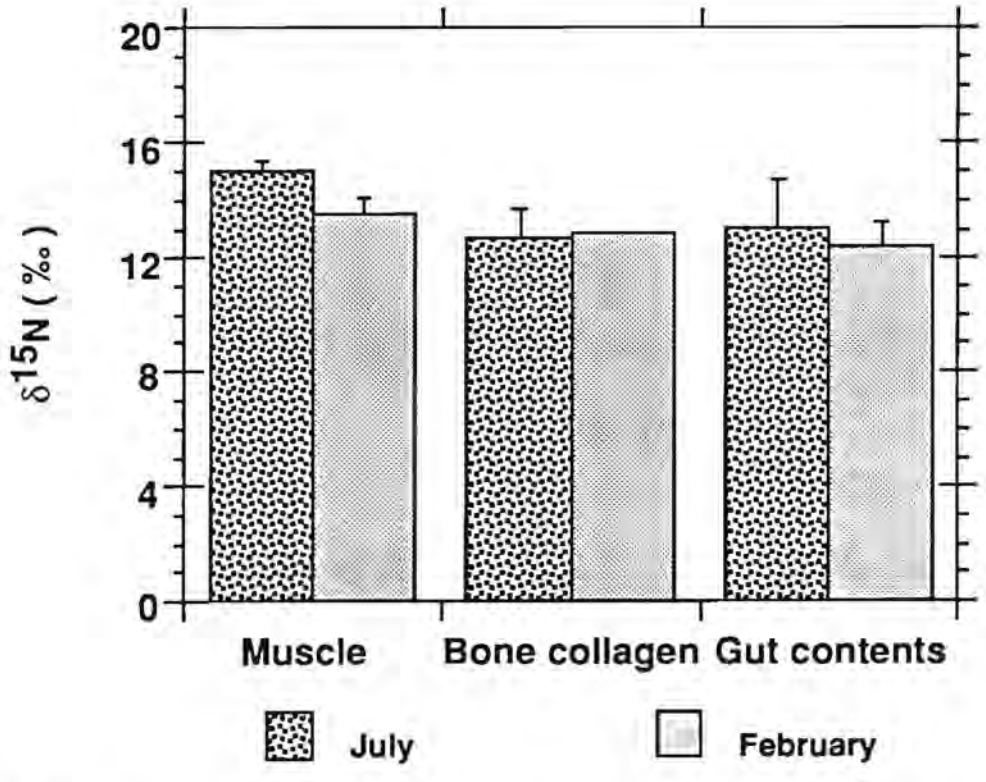
variations in the gut contents and cannibalism, depending on food availability. Hake may be cannibalistic (Payne *et al.* 1987). What effect cannibalism would have on isotope ratios is speculative, as the extent of cannibalism would have to be estimated. However some of the 'cannibalism' is on individuals of *Merluccius paradoxus*, and small fish of this species sampled as prey items for *M.capensis* do not appear to differ much in isotopic composition from *M.capensis*. The isotopic effect of cannibalism in the genus *Merluccius* would probably not differ as to which species was consumed by *M.capensis*. Trophic enrichment of isotope ratios in large hake may be due, to a large extent, on the consumption of other hake,

Slower turnover rate of the tissues of large hake may be the second reason for the lack of seasonal variation in large hake tissues. As small hake form an important part of the diet of large hake (Payne *et al.* 1987) it would be expected that the seasonal differences in $\delta^{13}C$ in small hake would be reflected in the tissues of the large hake. This is not the case. It therefore seems that the tissue turnover rate of the larger fish is not fast enough to reflect seasonal changes in $\delta^{13}C$. This is consistent with the findings of Simenstad and Wissmar (1985) who attribute the inconsistent and small seasonal variations of $\delta^{13}C$ in secondary consumers (certain zooplankton, crustacean and fish species) to differences in turnover rates of populations and tissue carbon.

Nitrogen

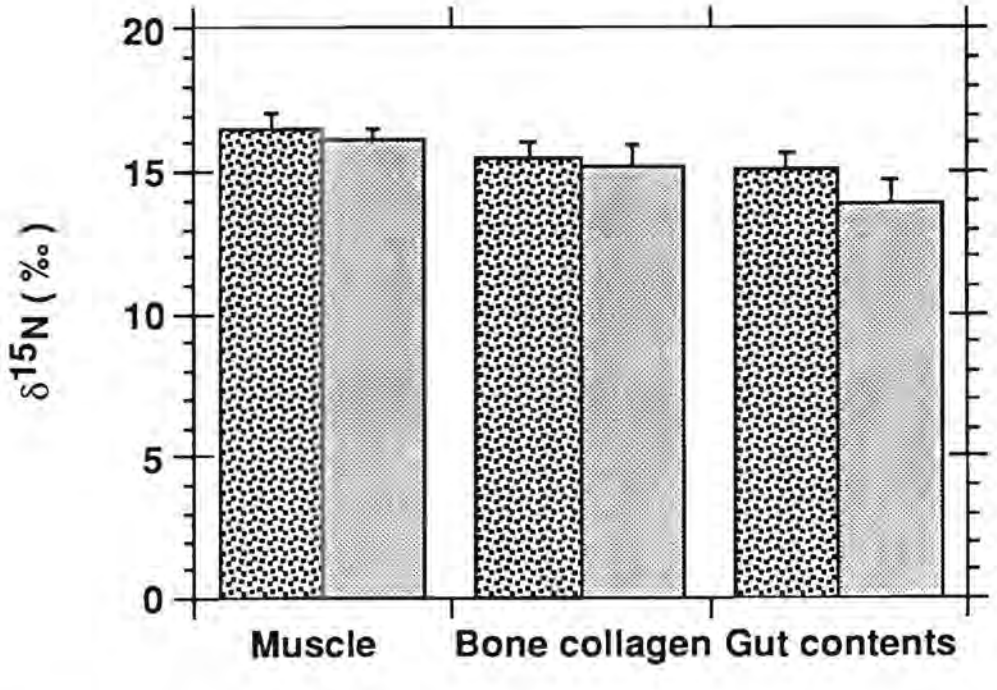
No seasonal differences are apparent in $\delta^{15}N$ in the zooplankton, and muscle, bone collagen and gut contents of small and large hake (Fig.4.1.4 and 4.1.5). There appears to be a seasonal difference in the zooplankton size class 200-500μm. However the sample size is too small to verify this statistically (see section 3.1, Table 3.1.4.).

Fig. 4.1.4.



$\delta^{15}N$ in the muscle, bone collagen and gut contents of small hake (<40cm) sampled in July 1990 and February 1992. Bars on histograms indicate standard error.

Fig 4.1.5.



$\delta^{15}N$ in the muscle, bone collagen and gut contents of large hake (≥40cm). Figure legends are the same as those for Fig. 4.1.4.

Seasonal variability in $\delta^{15}N$ has been reported in suspended particles in a warm core ring (Altabet and McCarthy 1985) and in particles in the deep Sargasso sea (Altabet and Deuser 1985). Cifuentes *et al.* (1988) found seasonal variability in $\delta^{15}N$ in suspended particulate matter in the Delaware estuary. Values ranged from 5.5 to 12.2$^{o}/_{oo}$ during winter, 2.3$^{o}/_{oo}$ was measured in early spring with a late spring maximum of 18.7$^{o}/_{oo}$, and two values (8 and 10 $^{o}/_{oo}$) were measured in summer (Cifuentes *et al.* 1988). The light spring $\delta^{15}N$ value was associated with fractionation of ammonium at concentrations greater than 20μm. The 18$^{o}/_{oo}$ maximum three weeks later was attributed to the use of a pool of NH_4^+ by phytoplankton that was enriched due to previous fractionation during nitrogen assimilation (Cifuentes *et al.* 1988).

$\delta^{15}N$ of nitrate dissolved in Pacific deep water is 6-8$^{o}/_{oo}$ (Miyake and Wada 1967, Wada and Hattori 1976). Deep, nutrient rich water is brought into the euphotic zone by upwelling (Brown and Hutchings 1987). $\delta^{15}N$ of suspended particles increases as the concentration of nitrate declines, due to fractionation during assimilation (Checkley and Miller 1989). $\delta^{15}N$ ranges from -3 to 13$^{o}/_{oo}$ in euphotic waters where no detectable nitrate is present (Wada 1980, Saino and Hattori 1980, 1985, 1987, Altabet and McCarthy 1985, Altabet 1988 in Checkley and Miller 1989). In waters where ammonium is the primary nitrogenous nutrient available due to scarcity of nitrate, zooplankton is depleted in $\delta^{15}N$ compared to zooplankton in eutrophic waters. This is possibly due to the increased importance of autotrophs that fix 'new' N_2 with little or no fractionation (Saino and Hattori 1978, 1980, 1985, 1987 in Checkley and Miller 1989).

It would appear on the west coast that $\delta^{15}N$ should vary not only with season but possibly also with upwelling pulses and nutrient availability. Therefore, it is not possible to draw any conclusions about seasonal variability of $\delta^{15}N$ from plankton sampled once in February and once in July. Sampling over a much shorter time period would be necessary. This conclusion is further supported by the lack of variation in $\delta^{15}N$ in fish tissues. In the large fish this could again be attributed to a slow tissue turnover time. The tissue turnover rate sufficient to indicate

$\delta^{13}C$ changes with season in small fish may be too slow to indicate the shorter term variation of $\delta^{15}N$ due to upwelling pulses and changes in nutrient availability and type.

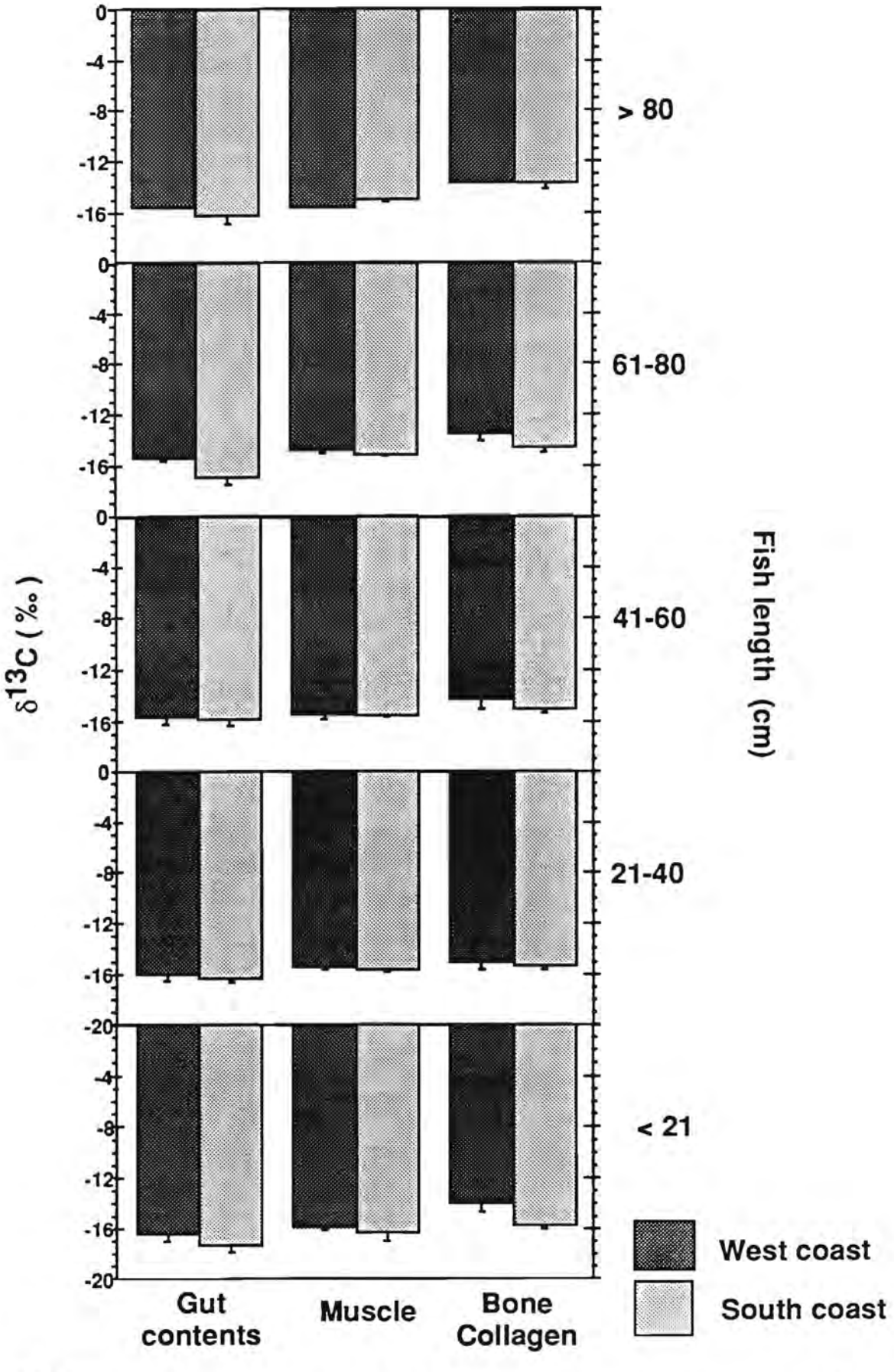
4.2 Geographical variation in $\delta^{13}C$ and $\delta^{15}N$

Carbon

Fig.4.2.1 shows $\delta^{13}C$ values for fish of different length classes sampled on the west and south coasts. While no statistically significant difference was found in $\delta^{13}C$ on the two coasts (see section 3.2), $\delta^{13}C$ is consistently slightly lower on the south coast than on the west coast. The seasonal differences in $\delta^{13}C$ is indicated in more enriched values when productivity was high (i.e. during upwelling cycles in winter). It is possible that the $\delta^{13}C$ values on the west coast are more enriched due to high phytoplankton productivity associated with upwelling, and plankton species composition. Diatoms are dominant in the phytoplankton on the west coast (Shannon and Pillar 1986, Brown and Hutchings 1987) and may cause enriched $\delta^{13}C$ values on the west coast. Copepods and euphausiids are found on the west coast, copepods being largely herbivorous and euphausiids omnivorous (Hutchings *et al.* 1991). However standing stocks of copepods are higher on the south coast, large calanoid copepods dominating the plankton (Hutchings *et al.* 1991). Euphausiids are present on the south coast, but their standing stock is lower than on the west coast (Hutchings *et al.* 1991). It would appear that at the level of the zooplankton there may be one more link in the food web on the west coast than on the south coast. This would primarily affect the isotopic values of the small fish ($\leq$20cm) as these feed mainly on zooplankton prey (Payne *et al.* 1987). This is evident in Fig.4.2.1 as the difference in $\delta^{13}C$ in the gut contents, muscle and bone collagen between the west and south coasts is larger in the $\leq$20cm size class than in any of the other size classes.

Sholto-Douglas (1992) found that pelagic fish and plankton sampled from the west coast were more enriched than samples from the Agulhas bank, indicating that fish on the Agulhas bank appeared to be consuming plankton one size class lower than fish on the west coast. This is consistent with the findings in hake. It would appear that small fish on the west coast consume mainly omnivorous euphausiids, and those on the south coast a higher proportion of

Fig. 4.2.1.



$\delta^{13}C$ in hake gut contents, muscle and bone collagen from the west and south coasts. Histogram bars indicate standard error.

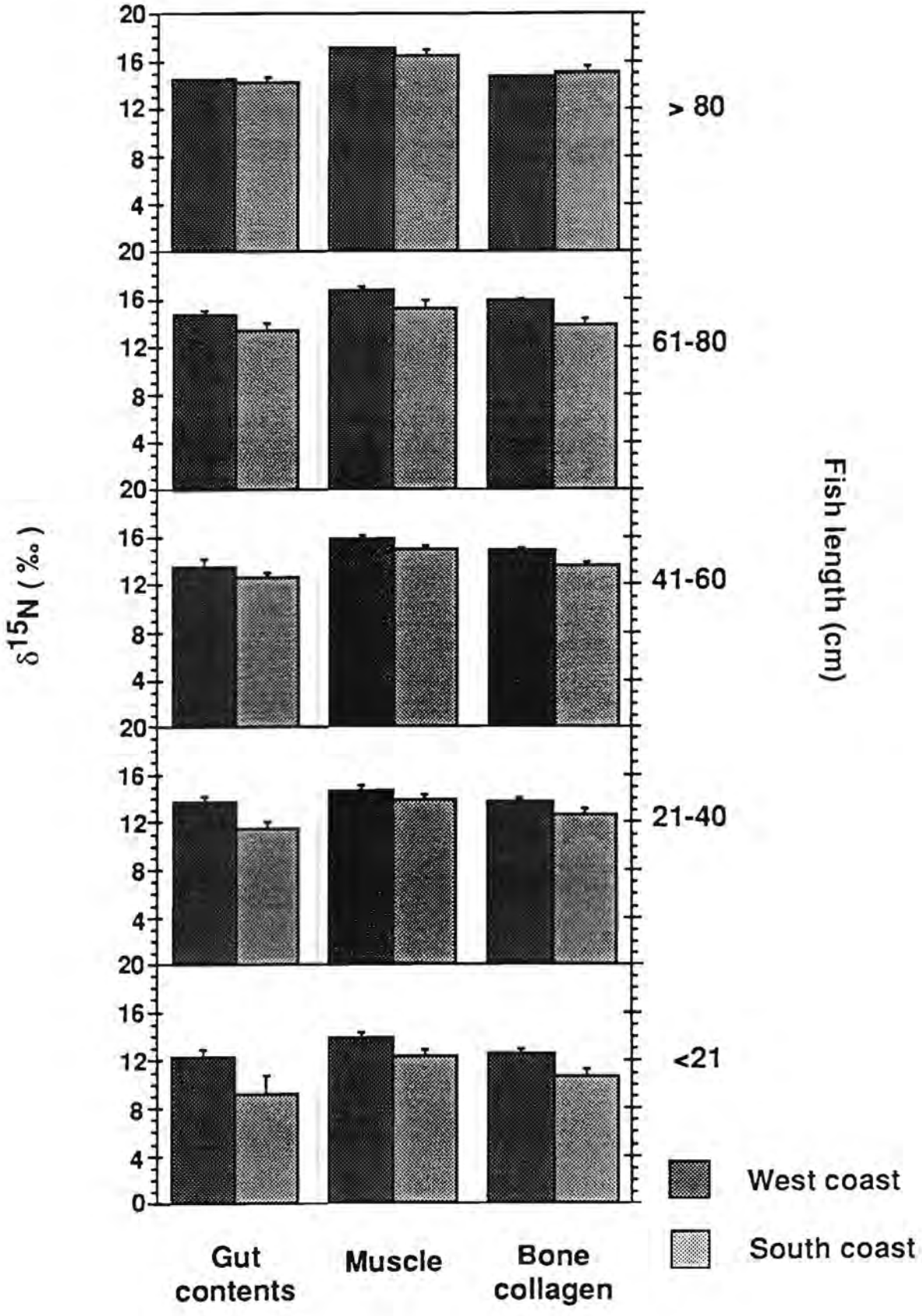
herbivorous zooplankton, causing depleted $\delta^{13}C$ values in fish tissues on the south coast. This difference may also be due to environmental factors affecting the stable isotope ratios of zooplankton differently in the separate areas, not detected in a study involving mainly fish. As large hake feed on pelagic fish and other hake, fractionation with trophic level may indicate the area in which the hake are feeding.

Nitrogen

The difference in $\delta^{15}N$ between the west and south coasts is statistically significant (see section 3.2), being enriched in ^{15}N on the west coast (Fig.4.2.2.). The reasons for the geographic difference in $\delta^{13}C$ already discussed apply to $\delta^{15}N$. It appears that the abundance of omnivores in the zooplankton on the west coast and herbivores on the south coast may account for the isotopic differences found on the two coasts.

Mullin *et al.* (1984) found lower $\delta^{15}N$ in zooplankton where the nutrient source was ammonium rather than nitrate. According to Probyn (1985), the most important nitrogen source in the southern Benguela system is nitrate (71%). The three size classes of phytoplankton sampled preferred ammonium as a nutrient source (Probyn 1985), plankton production in oceanic waters being supported mainly by regenerated nitrogen (Probyn 1985). Zooplankton in these waters are isotopically lighter than eutrophic water zooplankton (Mullin *et al.* 1984). Less upwelling on the south coast may mean that phytoplankton rely on regenerated nitrogen (ammonium) as a principal nitrogen source, possibly causing depletion in ^{15}N of plankton relative to the west coast, as zooplankton is depleted in ^{15}N where ammonium is the primary nitrogen source compared to zooplankton in eutrophic waters (Saino and Hattori 1978, 1980, 1985, 1987 in Checkley and Miller 1989). Phytoplankton have very rapid growth rates in upwelling areas, where blooms develop in days (Barlow 1982). In non-upwelling areas, blooms may take weeks to develop (Barlow 1982). Samples for comparison of $\delta^{13}C$ and $\delta^{15}N$ in plankton for seasonal and geographical differences would need to be undertaken regularly

Fig. 4.2.2.



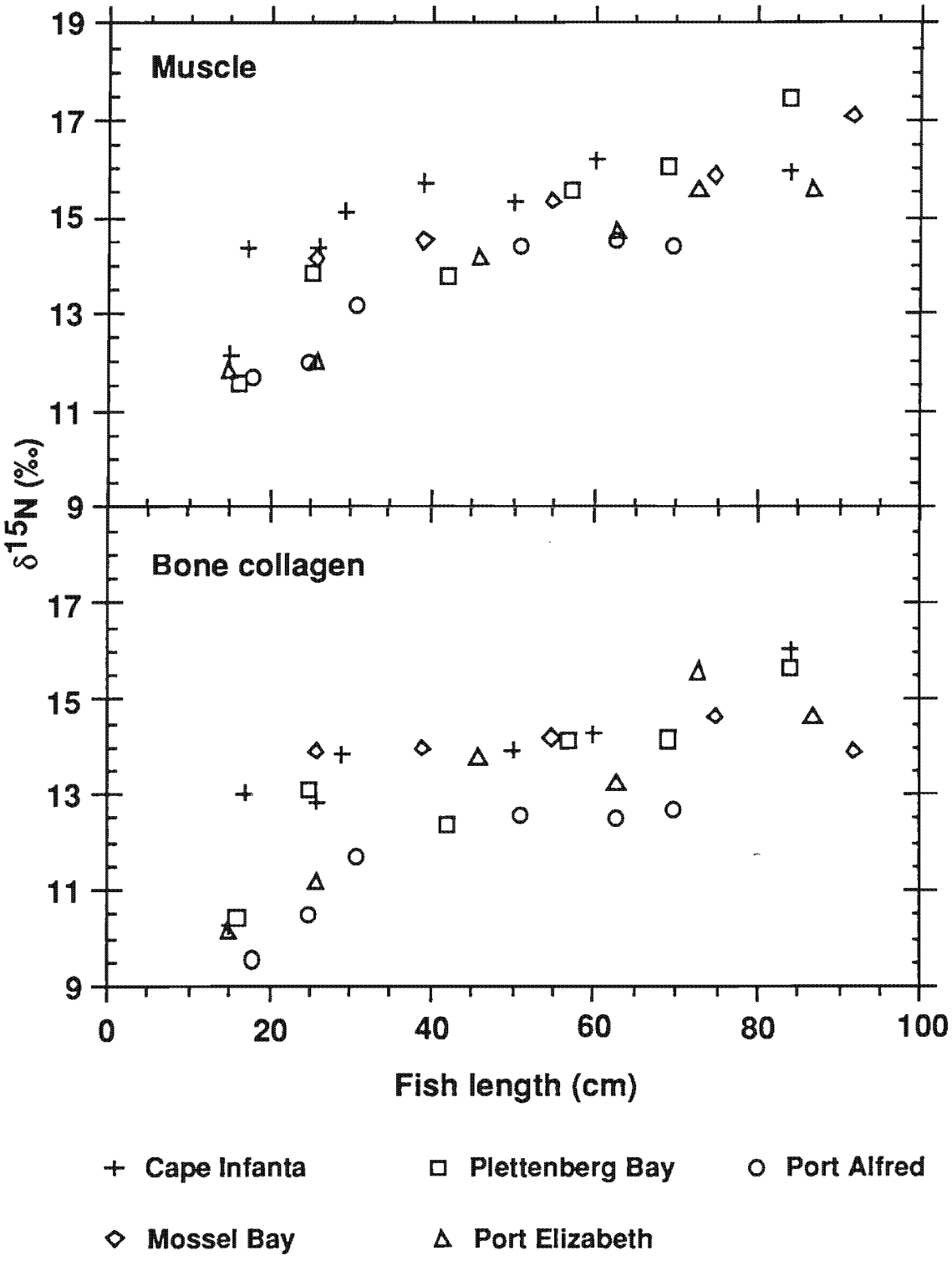
$\delta^{15}N$ in hake gut contents, muscle and bone collagen from the west and south coasts.

Histogram bars indicate standard error.

over a sufficient time period to determine short term isotopic variations which would affect secondary and tertiary consumers. It would seem that the above-mentioned difference in zooplankton species composition on the west and south coasts may be responsible for the higher $\delta^{15}N$ values on the west coast in muscle and bone collagen of *M.capensis*. Differences in $\delta^{15}N$ of available nutrients may also contribute to the geographic isotopic variation.

There does not appear to be much difference in $\delta^{15}N$ between the various west coast stations in hake muscle and bone collagen. However on the south coast, the $\delta^{15}N$ values at Port Alfred for muscle and bone collagen of all sizes of hake sampled appear to be lower than $\delta^{15}N$ at the other south coast stations sampled (Fig.4.2.3). Isotope values appear to become more depleted in ^{15}N from Cape Infanta along the coast to Port Alfred. The standing stock of copepods increases from west to east on the south coast (Peterson and Hutchings 1989, Hutchings *et al.* 1991). Swarms of euphausiids are found in the Agulhas current (Cornew *et al.* 1991), a lower standing stock being present on the Agulhas bank than on the west coast (Hutchings *et al.* 1991). The lower $\delta^{15}N$ values at Port Alfred may be associated with the increasing standing stock of copepods from west to east. Copepods do not appear to be present in the few gut content analyses undertaken in small hake on the south coast. This may be due to the 'snapshot', view of hake diet by gut content analysis. Small hake on the south coast appear to feed on other fish where crustacean prey is not available (Payne 1986). It is possible that the isotopic variability is caused by migration of prey fish. However the small, mainly planktivorous hake exhibit small differences in $\delta^{15}N$ with station area, suggesting that the isotopic variation between the two coasts in fish tissue may originate in the zooplankton prey. Although hake do not appear to feed on copepods, they may form a part of the diet of pelagic fish which are in turn consumed by hake. The difference in species composition of the zooplankton on the two coasts may have an indirect effect on ^{15}N and ^{13}C in the size classes of hake which consume fish.

Fig.4.2.3



$\delta^{15}N$ in muscle and bone collagen of hake sampled on the south coast to indicate isotopic site differences.

The lower $\delta^{15}N$ from Cape Infanta to Port Alfred does not only occur in the small fish. The difference is also manifest in large hake. Hake migrate vertically to feed (Botha 1973, 1980, Payne *et al.* 1987) and possibly to spawn (Botha 1980, Payne 1989). Hake migrate inshore in spring and offshore in autumn and winter (Roux 1949, Payne 1986). Evidence for alongshore migrations has not been reported. The isotopic data suggest that hake tend to migrate onshore and offshore in the same area, and do not seem to undertake alongshore migrations, since there is a clear isotopic gradient from west to east.

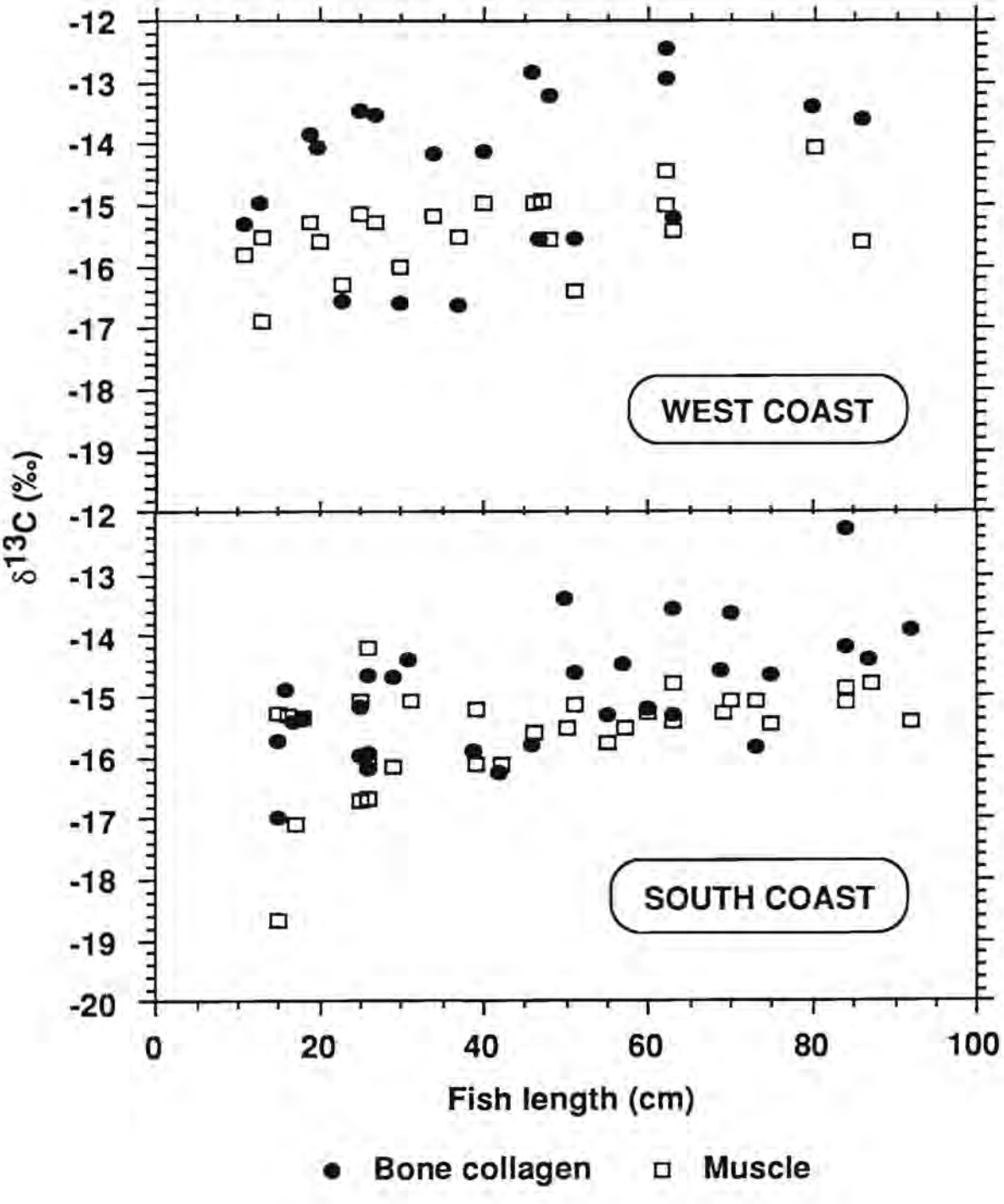
4.3. Variation of isotope ratios with fish length and tissue type

Carbon

Fish length

$\delta^{13}C$ does not vary significantly in hake bone collagen or gut contents with increasing fish length on either the west or south coasts. There is a significant increase in $\delta^{13}C$ in hake muscle with increasing fish length (Fig.4.3.1) (see Table 3.2.1). $\delta^{13}C$ appears to increase with increasing mass in Dover sole (Rau *et al.* 1981). Sholto-Douglas (1992) found that $\delta^{13}C$ in muscle and bone collagen of anchovy decreases with increasing fish length on the west coast although this was not statistically significant. $\delta^{13}C$ in the anchovy tissue values showed a stronger negative correlation with fish length when Agulhas bank samples were added to the west coast samples (Sholto-Douglas 1992). The negative correlation found was attributed to the fish possibly switching from a carnivorous to an omnivorous diet at a length of 80mm (Sholto-Douglas *et al.* 1991). Physiological, and not solely dietary causes for this effect may exist. A negative correlation between fish length and $\delta^{13}C$ in muscle and bone collagen would not be expected in hake as there does not seem to be any evidence of their feeding on a diet likely to be isotopically light as they increase in length. $\delta^{13}C$ in hake muscle and bone collagen in all samples analysed together appears to be too variable to make any deductions about trends with fish length (Fig.4.3.1). According to DeNiro and Epstein (1978), seasonal variation in the carbon isotopic composition of the diet may affect the accuracy with which the relationship between $\delta^{13}C$ of the animal and its diet can be tested. This may be the reason for the apparent variability in the $\delta^{13}C$ in hake muscle and bone collagen. It has been shown that $\delta^{13}C$ in the tissues of small hake varies seasonally (see section 4.1). As hake are opportunistic feeders (Payne *et al.* 1987) the isotopic variation may be due to changes in diet. However as dietary changes should also affect $\delta^{15}N$, and $\delta^{15}N$ does not vary in hake tissues with season, it is likely that the seasonal variation of $\delta^{13}C$ in hake muscle and bone collagen is possibly the cause of variation in the $\delta^{13}C$ samples.

Fig. 4.3.1



Comparison of $\delta^{13}C$ in muscle and bone collagen in hake sampled on the west and south coasts. $\delta^{13}C$ does not increase significantly with increasing fish length.

Tissue type

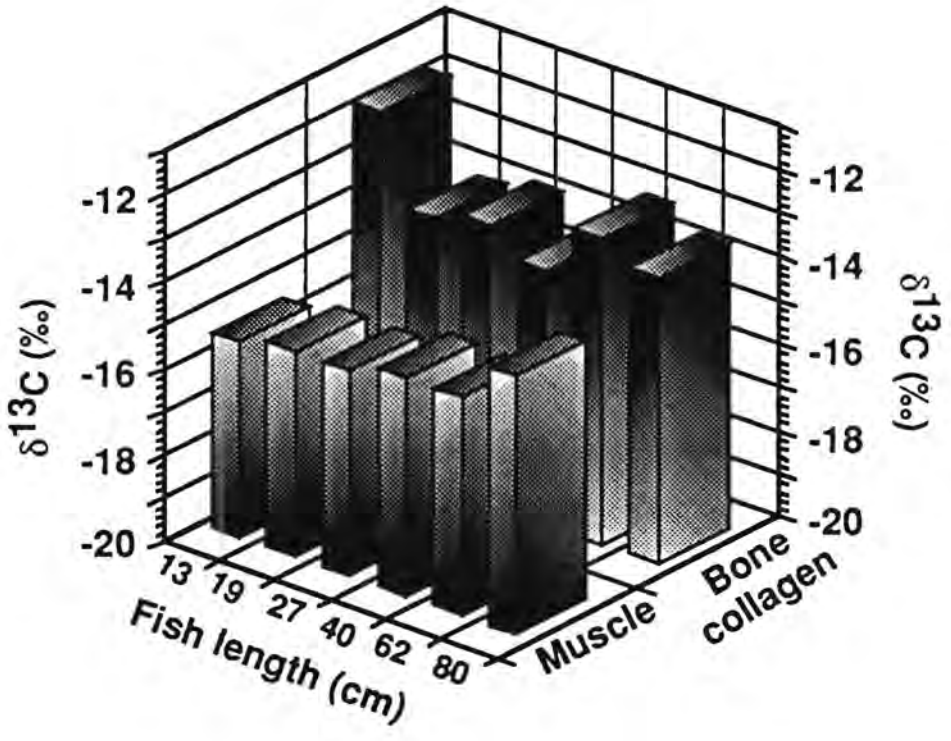
There is no statistically significant difference in $\delta^{13}C$ between muscle tissue and bone collagen (see section 3.3). However hake samples from Cape Columbine, collected in July 1990 have significantly higher $\delta^{13}C$ values in bone collagen than in muscle (Parkins, unpublished BSc (Hons) project) (Fig.4.3.2). It appears that seasonal variations in hake prey, and isotopic variation in individual fish may be responsible for the lack of significant differences in $\delta^{13}C$ between muscle and bone collagen on the west and south coasts. The higher $\delta^{13}C$ values in bone collagen when compared to muscle tissue in fish from the same area and season is consistent with isotopic values of pelagic fish (Sholto-Douglas *et al.* 1991). Lee-Thorp *et al.* (1989) found bone collagen to be enriched in $\delta^{13}C$ relative to muscle in terrestrial animals. According to Hare *et al.* (1991), synthesis of some amino acids in bone and muscle tissues seem to be catalysed by similar enzymatic reactions. It appears that muscle tissue acquires less ^{13}C than bone collagen, indicating differential fractionation of ^{13}C into muscle and bone collagen (Sholto-Douglas *et al.* 1991). $\delta^{13}C$ of dietary glycine in pigs is $8^{o}/_{oo}$ higher than the isotopic value of the diet (Hare *et al.* 1991). As glycine is the most abundant amino acid in bone collagen (Hare *et al.* 1991), the mean $\delta^{13}C$ may be elevated. This would be depend on the amino acid composition of hake muscle, which is different to that of pigs. The same pattern would, however be expected to apply.

Nitrogen

Fish length

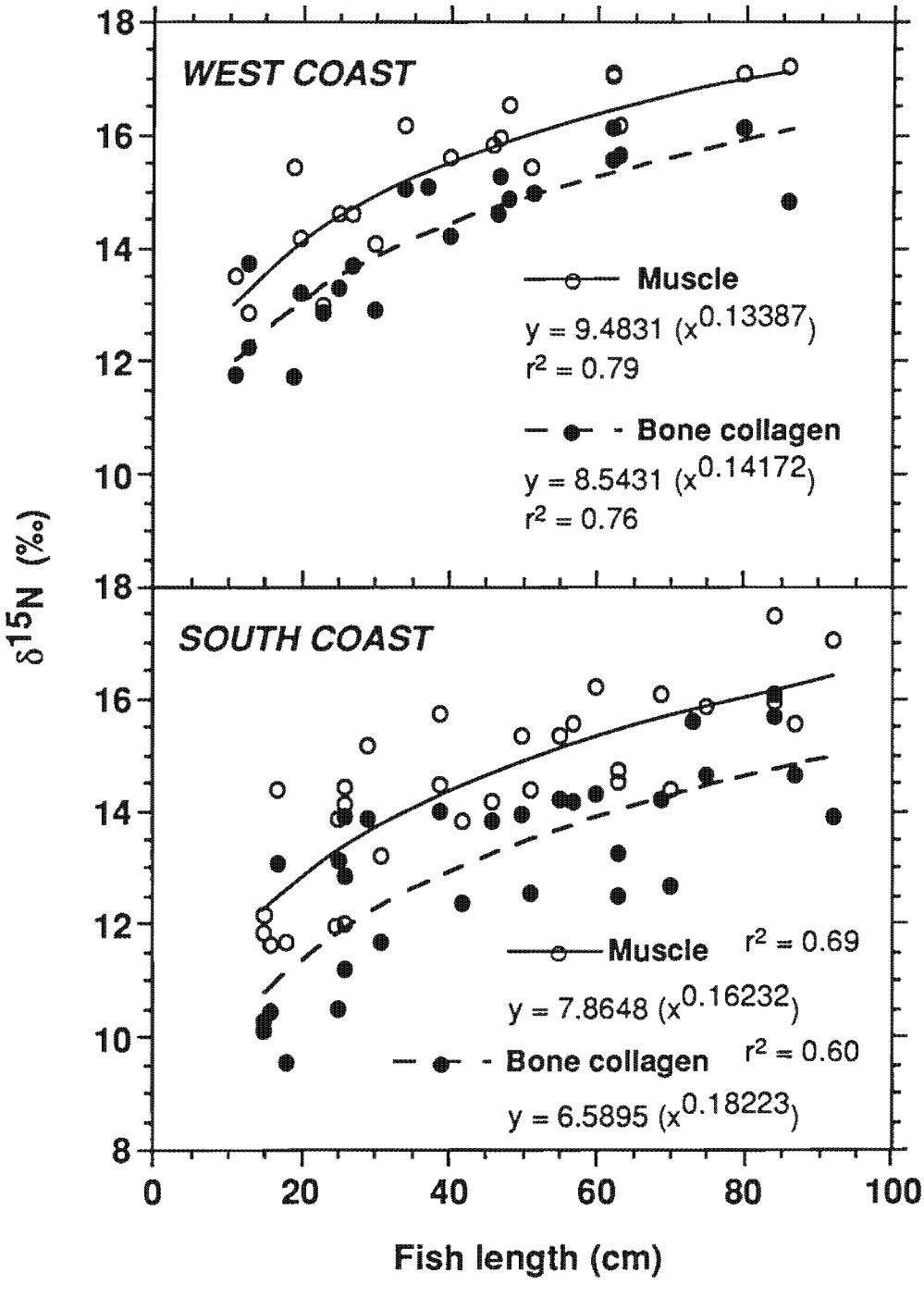
$\delta^{15}N$ in muscle tissue and bone collagen increases with increasing fish length. The relationship is non-linear, power curves best fitting the data points (Fig. 4.3.3). Small animals have faster metabolic rates than large animals (Schmidt-Nielsen 1984), juvenile fish having faster metabolic rates than adults (Panadian 1987). Furthermore, the metabolic rates of bathypelagic fish decrease with increasing depth (Panadian 1987). As large hake are found in deeper water than small hake, this may further account for what appears to be increased fractionation of

Fig.4.3.2



Comparison of $\delta^{13}C$ between muscle tissue and bone collagen in hake of different lengths, sampled in the Cape Columbine area on the west coast.

Fig. 4.3.3.



Variation in $\delta^{15}N$ with increasing fish length in samples from the west and south coasts. Power curves fitted to the data are shown.

$^{15}N/^{14}N$ in small fish, the slower metabolism of the large fish causing more integration of isotopic nitrogen. According to Botha (1985) mature hake are generally defined as those being more than 40cm in length. Payne (1986) found that *M.capensis* on the south coast begin to mature at a length of 22cm, reaching 50% maturity at 53cm. Males mature earlier than females (Payne 1986). Mature animals incorporate some of their dietary intake into gonad development and reproductive output (Schmidt-Nielsen 1984), whereas small animals use their energy intake for growth. This may also cause different isotopic fractionation in fish of different levels of maturity.

Sholto-Douglas *et al.* (1991) found a negative correlation in $\delta^{15}N$ in pelagic fish with increasing fish length. This was attributed to large fish possibly feeding on an isotopically lighter diet, although physiological causes were not ruled out (op.cit.). It is likely that the increase in $\delta^{15}N$ with increasing fish length in hake is to a large extent dietary. Small hake feed mainly on plankton, and large hake on other fish. Crustaceans are also an important part of the hake diet (Payne *et al.* 1987). The large fish therefore sometimes feed at the same trophic level as the small fish. As hake can be cannibalistic (Payne *et al.* 1987) they may feed on the small hake, or on pelagic fish which consume a similar diet to the small hake, thereby feeding at a higher trophic level. If large hake were to feed continuously on the same trophic level as small hake, $\delta^{15}N$ in the tissues of fish of various sizes would be similar, although a small difference due to the faster metabolic rate in small fish would be observed. The increase in $\delta^{15}N$ in hake tissues with increasing length shows that the majority of ^{15}N integrated into the tissue of large hake has its origin at a higher trophic level than that in small hake. As the power curves fitted to the isotope data (Fig.4.3.3) do not flatten suddenly, it appears that as hake get larger, they feed on prey from higher trophic levels or incorporate less plankton into their diet.

Tissue type

$\delta^{15}N$ in hake muscle is 1-1.5$^{o}/_{oo}$ higher than that of bone collagen (Fig.4.3.3). During nitrogen metabolism, more ^{15}N appears to be incorporated into muscle tissue than bone collagen, the opposite to carbon isotopic fractionation. Muscle tissue is also more enriched in ^{15}N than bone collagen in pelagic fish (Sholto-Douglas *et al.* 1991). On the west and south coasts, Sholto-Douglas *et al.* (1991) found that $\delta^{15}N$ in bone collagen showed a stronger negative correlation with fish size than muscle tissue. In hake, the shape of the power curves fitted to the isotopic data (Fig.4.3.3) appears to be similar for muscle tissue and bone collagen. This suggests some similarity in the process of nitrogen isotopic incorporation in the tissues. The difference in $\delta^{15}N$ between the two coasts is also evident in Fig. 4.3.3. The shape of the power curves appear to indicate similar processes of isotopic nitrogen incorporation into the tissues, even though the $\delta^{15}N$ values are lower on the south coast.

Individual amino acids are enriched in $\delta^{15}N$ relative to dietary amino acids in rat liver (Gaebler *et al.* 1966). The isotopic enrichment was greatest for non-essential amino acids than essential amino acids (Gaebler *et al.* 1966). Synthesis of glutamate, aspartate, threonine and serine in muscle tissue and bone collagen of pigs appeared to have similar isotopic compositions, suggesting that the synthesis of these amino acids is catalysed by similar enzymatic reactions (Hare *et al.* 1991). $\delta^{15}N$ values for other amino acids in pig muscle were not available. However, amino acid catabolism differs between carnivorous fish and omnivorous terrestrial mammals (Cowey and Sargent 1979). Fish require almost twice the amount of amino acids as omnivorous land animals, although the net retention of protein by fish is not greater than that of mammals (Cowey and Sargent 1979). The proportions of different amino acids in hake bone collagen and muscle, and their specific isotope ratios may well explain the different $\delta^{15}N$ values of the two tissues. This would needs to be tested in a separate dietary study.

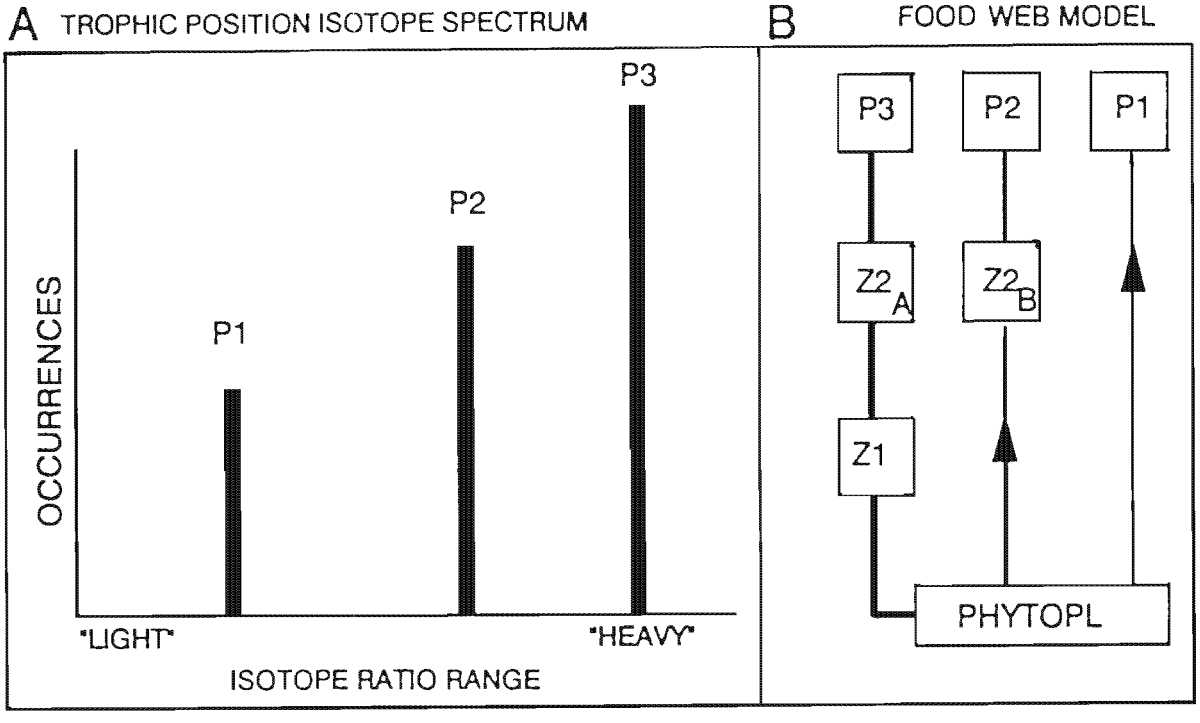
4.4 THE TROPHIC POSITION ISOTOPE SPECTRUM

The trophic position isotope spectrum (T.P.I.S) was developed by Monteiro *et al* (1991) to attempt to define and quantify carbon and nitrogen flow from fixation by primary producers to higher trophic levels, as an alternative to simple isotopic models of food chains. $\delta^{13}C$ dietary pathways of anchovy from the Southern Benguela were analysed in this way (Monteiro *et al.* 1991, Sholto-Douglas 1992). The model assumes that each $\delta^{13}C$ or $\delta^{15}N$ value represents the dietary history of the animal integrated over the turnover time of the particular tissue analysed. Variation in the dietary history of individual organisms is distinguishable (Monteiro *et al.* 1991). The distribution and frequency of occurrence of $\delta^{13}C$ and $\delta^{15}N$ reflects the pathway along which the carbon or nitrogen reached the particular trophic level where it is sampled. Fig. 4.4.1 depicts the hypothesis of the T.P.I.S. P1, P2 and P3 show the isotope ratios for each pathway, the height of the bar indicating the number of individuals that have fed by that pathway. P2 may indicate a mixed diet, where an organism feeds from P1 and P3. Whether P2 is a mixed diet or a separate food source would have to be estimated by gut content analysis (Monteiro *et al.* 1991). In this study $\delta^{13}C$ and $\delta^{15}N$ in hake muscle, bone collagen, gut contents, and prey items on the west and south coasts are represented in the T.P.I.S. and an attempt is made to define feeding pathways.

Carbon

Fig.4.4.2 shows the $\delta^{13}C$ values from the west coast samples depicted in the T.P.I.S. Values are indicated for all sizes of fish. As no $\delta^{13}C$ differences were found in muscle and bone collagen of hake of different sizes, size classes are not shown. $\delta^{13}C$ of hake gut contents and prey fish samples appear to correspond. The gut contents appear to be isotopically indicative of the prey fish consumed. This may be useful in assessing whether unidentifiable prey items in the fish gut are possibly part of the usual diet of the fish. Slight ^{13}C enrichment occurs from the gut contents and prey fish to hake muscle and bone collagen. The difference is small, and

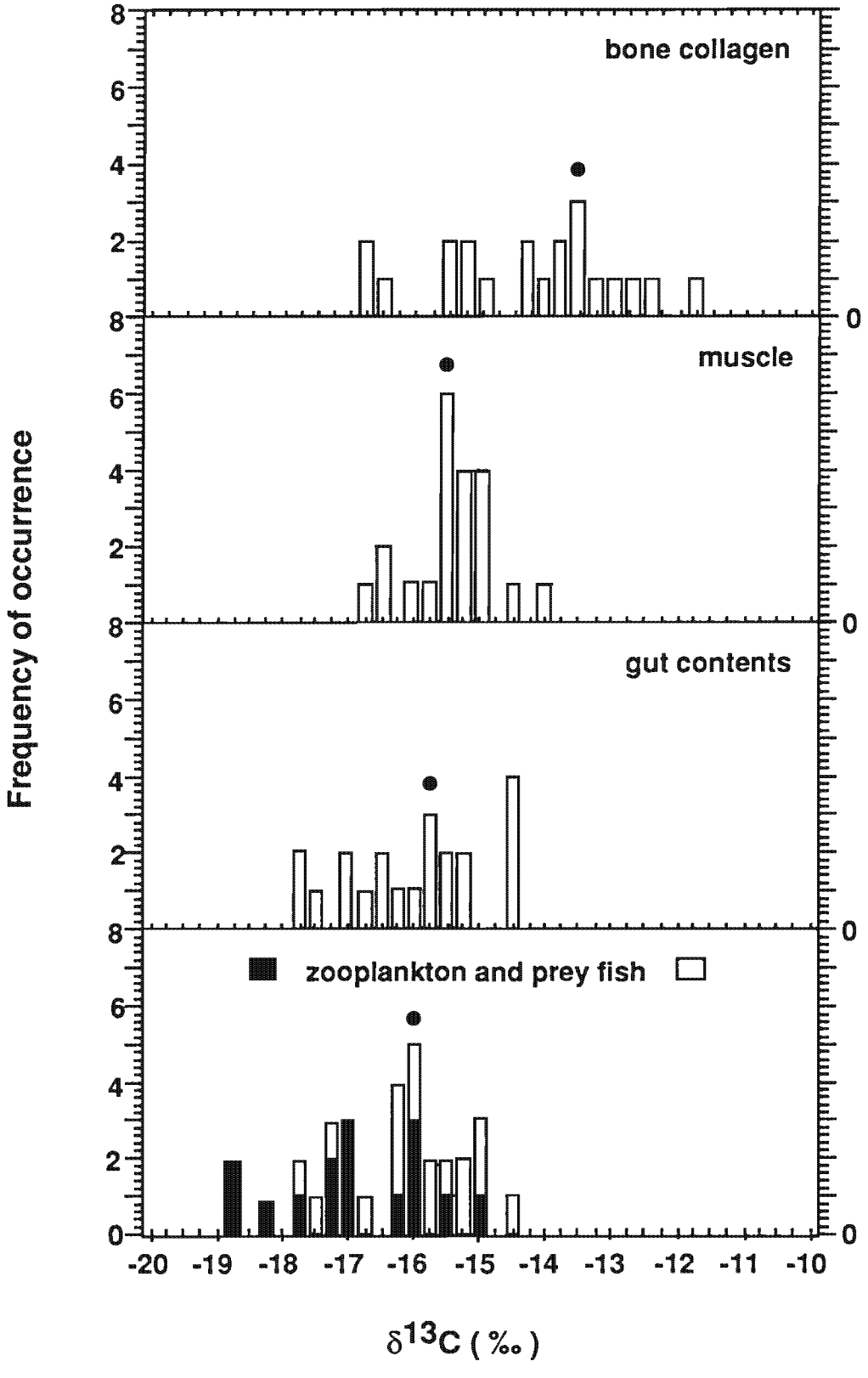
Fig. 4.4.1.



(After Monteiro *et al.* 1991)

The concept of the Trophic Position Isotope Spectrum is illustrated in A. P1, P2 and P3 are stable isotope ratios which characterise dietary pathways. These are a function of the number of intermediate steps in the trophic pathway. The height of the bars indicates the number of individuals which have fed via that pathway. P2 could be a mixture of P1 and P3, or an independant dietary pathway (tested by gut content analysis). B shows the relationship of the T.P.I.S. to a hypothetical pelagic food web. Phytopl. is phytoplankton, Z1 and Z2 are zooplankton categories. Z1 and Z2B represent herbivores, and Z2A predators.

Fig. 4.4.2.



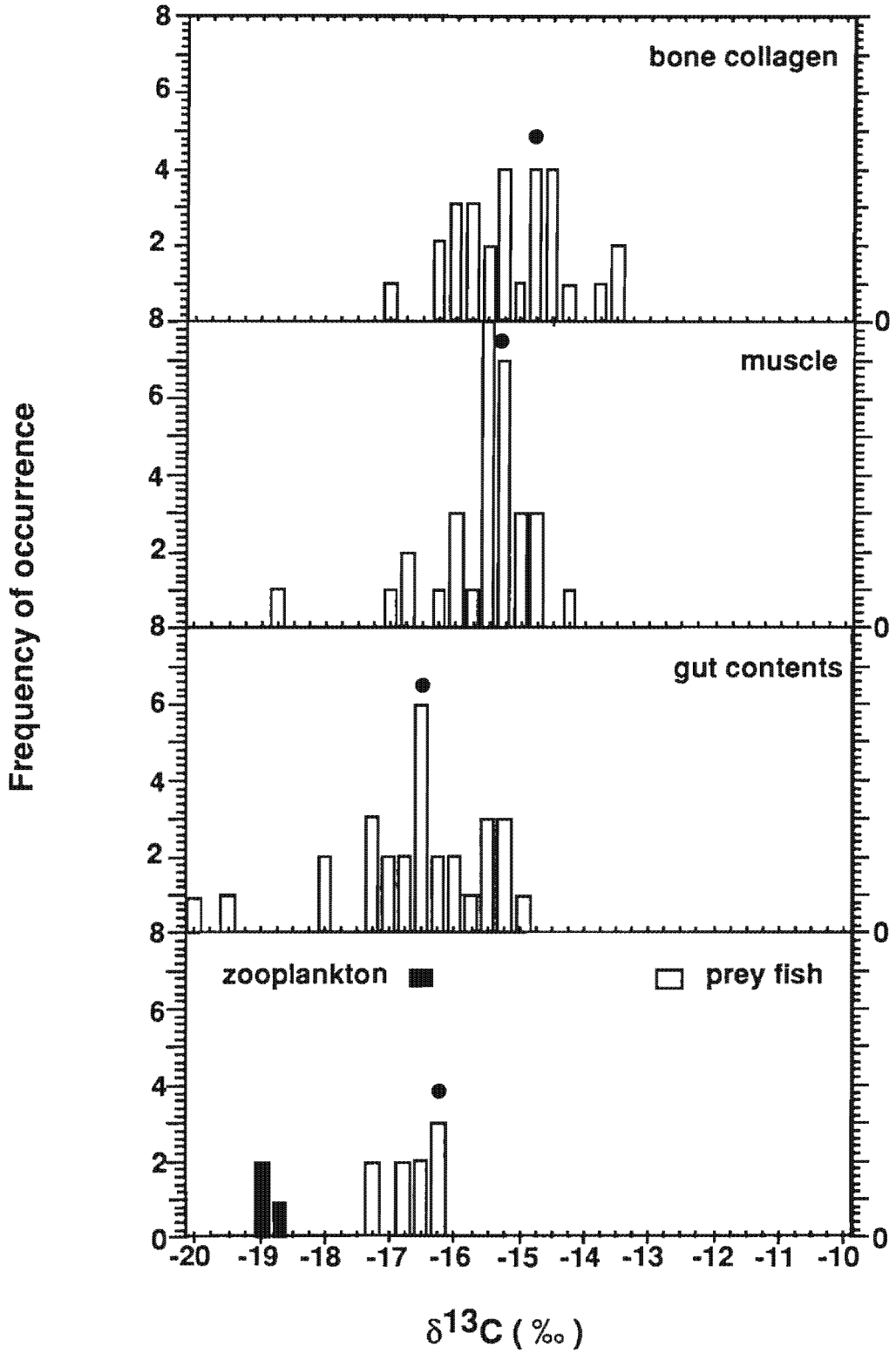
$\delta^{13}C$ in hake bone collagen, muscle and gut contents, prey fish and zooplankton from the west coast represented in the Trophic Position Isotope Spectrum. Closed circles (•) denote the highest occurrences, indicating the dominant isotopic feeding pathway from prey and gut contents to hake tissues.

not easily quantified due to scatter in the data. The feeding pathway indicated in Fig. 4.4.2. is therefore approximate. $\delta^{13}C$ in bone collagen appears to be more variable than in bone collagen than in muscle tissue, and fractionation of carbon between gut contents and bone collagen is greater than between gut contents and muscle tissue. Carbon fractionation between gut contents and muscle and bone collagen is higher in pelagic fish, and two feeding pathways are defined on the west coast (Sholto-Douglas 1992).

Fig.4.4.3 shows the T.P.I.S of south coast $\delta^{13}C$ values. The prey fish again appear to be isotopically included in the diet. The zooplankton $\delta^{13}C$ values do not appear to correspond with the gut content isotope values. The particular fish sampled may not have eaten the zooplankton which was analysed. Fractionation of carbon between the peak $\delta^{13}C$ values of the gut contents and muscle tissues seems to be slightly higher than on the west coast. $\delta^{13}C$ of bone collagen again appears to be variable. Differences in $\delta^{13}C$ between muscle tissue and bone collagen are small, bone collagen having slightly more ^{13}C than muscle tissue. Again the pathway indicated in Fig. 4.4.3 is approximate, as on the west coast.

The trophic pathways indicated by the T.P.I.S. in pelagic fish (Monteiro *et al.* 1991, Sholto-Douglas 1992) are more clearly evident than in $\delta^{13}C$ of hake tissues since more scatter is evident in the hake isotope data. A seasonal effect may be partially responsible. Alongshore migrations of pelagic fish, and the presence of red and white muscle in pelagic fish may also contribute to this difference. Fry (1988) notes that the greatest fractionation in $\delta^{13}C$ appears early in the food web. This appears to be the case in hake samples. Small hake feed mainly on zooplankton, while large hake are mainly piscivorous (Payne *et al.* 1987). These different pathways are not clear in the carbon T.P.I.S., although isotopic enrichment with increasing fish size and presumably trophic level is shown.

Fig. 4.4.3.



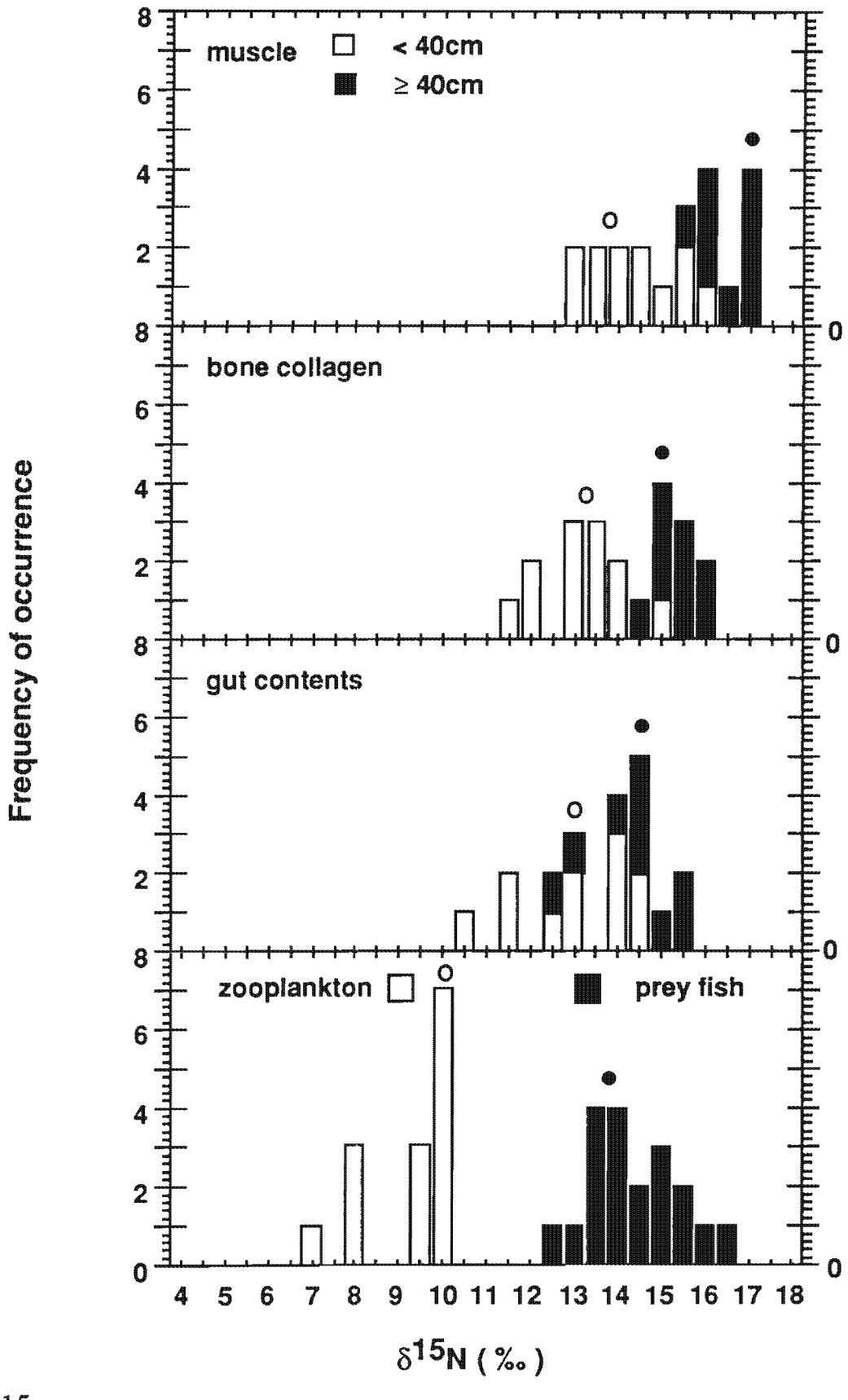
$\delta^{13}C$ in hake bone collagen, muscle and gut contents, prey fish and zooplanktonfrom the south coast represented in the Trophic Position Isotope Spectrum. Closed circles (•) denote the highest occurrences, indicating the dominant isotopic feeding pathway from prey and gut contents to hake tissues.

Nitrogen

Fig. 4.4.4 shows the T.P.I.S of the $\delta^{15}N$ values of west coast samples. The zooplankton with the lowest $\delta^{15}N$ values do not appear to be represented in the hake diet as a dominant food source. According to Sholto-Douglas (1992) there is a positive correlation between $\delta^{15}N$ and zooplankton size. It appears that only large zooplankton form a substantial part of the diet of small hake. This may be partly due to the high euphausiid biomass on the west coast. Enrichment of $\delta^{15}N$ with trophic level is evident from the T.P.I.S. peak frequency occurrence values of gut contents to muscle and bone collagen. The gut contents of the large fish appear to be enriched in ^{15}N relative to the small fish, although this may occur at the extremes of the size ranges. More fish samples with a finer division of size classes would be necessary to demonstrate this effect. $\delta^{15}N$ of the gut contents only indicates the ^{15}N source of the immediate past which has not yet been integrated into the hake tissues. According to DeNiro and Epstein (1981) consumers are enriched by 3-4$^{o}/_{oo}$ in ^{15}N relative to their diet. Hake muscle appears to be enriched by 2-3$^{o}/_{oo}$ in $\delta^{15}N$ relative to the gut contents in small and large fish. The isotopic enrichment between the gut contents and bone collagen is less than that between gut contents and muscle. The T.P.I.S. shows two peaks in $\delta^{15}N$ in bone collagen, indicating the two isotopic dietary pathways of small and large fish. These pathways are not as evident in the muscle tissue as there appears to be more isotopic variation in $\delta^{15}N$ of muscle tissue of small hake. $\delta^{15}N$ in individuals feeding on the same diet can be different (DeNiro and Epstein 1981). This appears to be the case particularly in small hake.

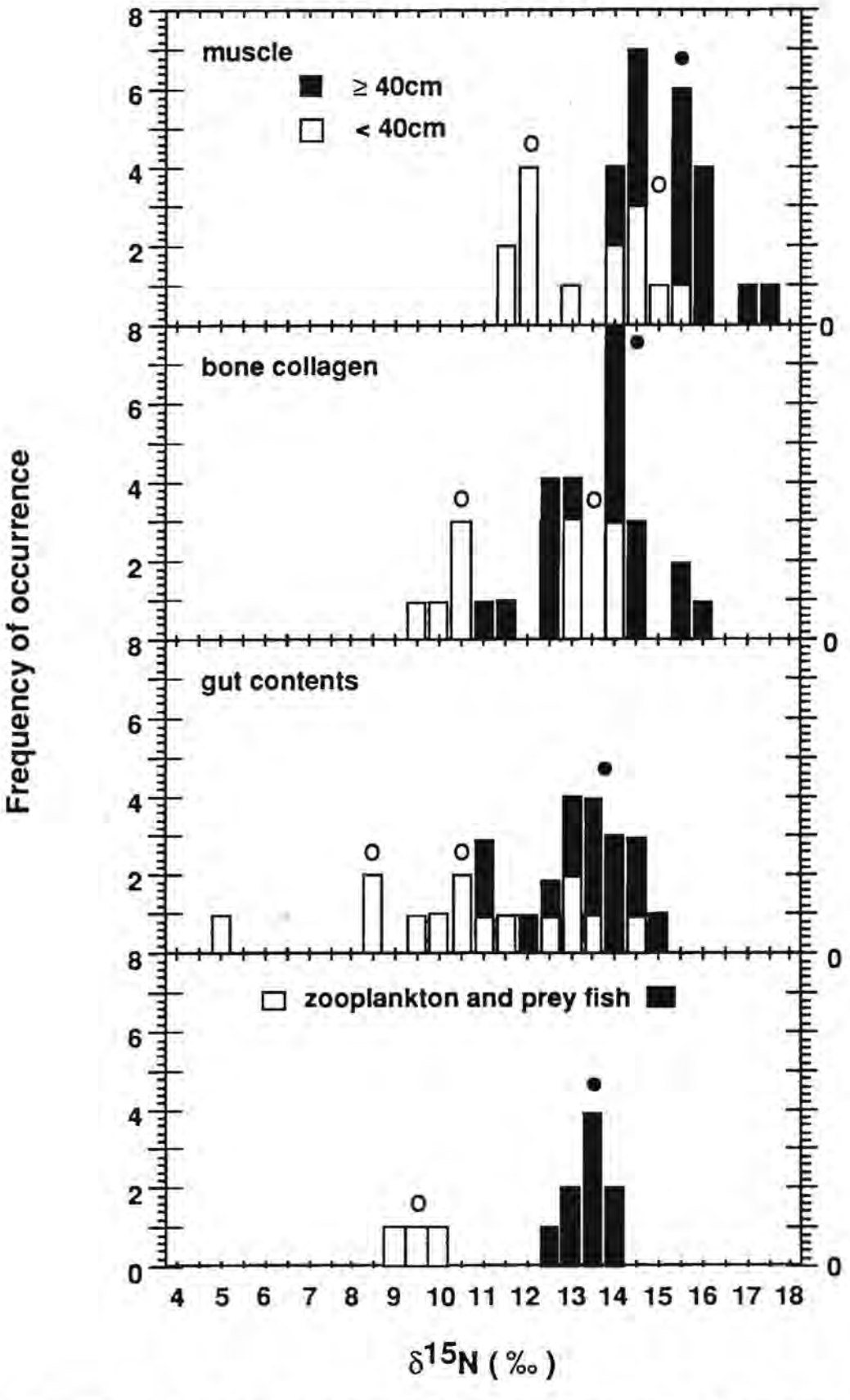
The T.P.I.S. of $\delta^{15}N$ from the south coast is shown in Fig. 4.4.5. All zooplankton and prey fish sampled appear to be isotopically represented in the gut contents. This may be due to the fish feeding on a wider range of plankton size classes than on the west coast. The $\delta^{15}N$ values of the gut contents are more variable on the south coast (5 -15$^{o}/_{oo}$) than on the west coast (10.5-15$^{o}/_{oo}$), suggesting that hake feed on a greater variety of organisms on the south coast. According to Payne (1986), more fish species are present on the south coast than on the west

Fig. 4.4.4.



$\delta^{15}N$ in hake muscle, bone collagen and gut contents, prey fish and zooplankton from the west coast, represented in the Trophic Position Isotope Spectrum. Closed circles (●) indicate the isotopic feeding pathways of large fish (≥40cm), and open circles (o) indicate the isotopic feeding pathways of small fish (<40cm).

Fig. 4.4.5.



$\delta^{15}N$ in hake muscle, bone collagen and gut contents, prey fish and zooplankton from the south coast, represented in the Trophic Position Isotope Spectrum. Closed circles (•) indicate the isotopic feeding pathway of the large hake (≥40cm), and the open circles (o) indicate both isotopic feeding pathways of the small fish (<40cm).

coast. Hake may have a wider range of available prey on the south coast. Small fish appear to obtain their dietary carbon from two isotopically distinct sources, one more enriched in ^{15}N than the other. These two nitrogen isotope dietary pathways are shown in Fig.4.4.5. This is not observed in the west coast hake samples. Fractionation of $\delta^{15}N$ between gut contents and muscle and bone collagen appears to be similar to the values found on the west coast. The different isotopic pathways of small and large fish are again indicated more clearly in $\delta^{15}N$ of bone collagen than muscle. The dietary pathways appear to have consistently higher $\delta^{15}N$ values on the west coast. The $\delta^{15}N$ values in the muscle and bone collagen of the west coast hake appear to indicate that large fish have consistently higher isotope ratios than small fish. $\delta^{15}N$ in small fish appears to be more variable on the south coast. This may be due to higher levels of cannibalism on the west coast which may cause the larger fish to have consistently higher levels of ^{15}N than the small hake on which they are feeding.

The pathways of dietary nitrogen in hake appear to be better represented in the T.P.I.S. than carbon. Fry (1988) suggests than ^{15}N is a more reliable trophic indicator than ^{13}C, particularly when analysing the diets of organisms high up in the food web. Owens (1987) suggests a trend of increasing $\delta^{15}N$ values in higher trophic levels. This appears to be the case in hake. The scenario is further complicated by different sizes of fish feeding on different trophic levels. The lower $\delta^{15}N$ in the tissues of hake on the south coast may be due to hake feeding on a larger variety of organisms and sizes of plankton. It appears that hake on the west coast feed on the zooplankton most enriched in $\delta^{15}N$, and by implication the largest of the plankton sampled. This may be due to the high euphausiid biomass on the west coast. On the south coast, where the euphausiid biomass is lower, hake appear to feed over a wider range of zooplankton size classes and prey fish, incorporating organisms depleted in $\delta^{15}N$ into their diets, not eaten by west coast hake or prey fish. The greater occurrence of piscivory on the south coast noted by Payne (1986) may be partly due to the lower euphausiid biomass on the south coast. The T.P.I.S. is an alternative way of utilising stable isotope ratio data, and is effective in clarifying dietary pathways not evident when using measures of central tendency.

4.5 THE TROPHIC POSITION OF HAKE

Hake are both predators and prey in the marine environment (Payne *et al.* 1987). Snoek (*Thrysites atun*)(Nepgen 1979 in Payne *et al.* 1987), kingklip (*Genypterus capensis*) (Macpherson 1983 in Payne *et al.* 1987), sharks and seals (Rand 1959 in Payne *et al.*1987) consume hake on the west coast. As hake are dominant in their habitat, a major predator is also other hake (Botha 1980), since intraspecific predation is often a function of density (Polis 1981). While cannibalism maintains the population size below the carrying capacity of the environment, ecological efficiency of secondary production is reduced (Polis 1981). Trophic levels are difficult to conceptualise when cannibalistic organisms are involved (Polis 1981). As large *M.capensis* are found in the same depths as small *M.paradoxus* (Botha 1973), large *M.capensis* consume small *M.paradoxus* individuals. True cannibalism is more prevalent in large *M.paradoxus* (Payne *et al.*1987).

Muscle and bone collagen of small hake are depleted in $\delta^{15}N$ by 2-4$^{o}/_{oo}$ relative to large hake (see section 4.4), indicating that *M.capensis* does not appear to feed at one trophic level. Small hake feed mainly on zooplankton, particularly euphausiids on the west coast (Payne *et al.* 1987). Trophic levels may exist in different size classes of zooplankton, causing isotopic variation in the prey of the small hake and pelagic fish on which large hake may feed. Large hake feed on crustaceans as well as demersal and pelagic prey (Payne *et al.* 1987). The positive correlation in $\delta^{15}N$ with increasing hake length indicates that the diet of large fish appears to be mainly piscivorous. The opportunistic feeding behaviour of hake is evident. Cannibalism in hake probably varies with population size and prey availability. Changes in hake abundance may affect the abundance of predators or prey, as appears to occur in the Pacific whiting, *Merluccius productus* (Livingston and Bailey 1985). According to Ware (1992), hake are tertiary predators. This appears to be true for large hake, as their muscle tissue and bone collagen are enriched in $\delta^{15}N$ by 2-4$^{o}/_{oo}$ relative to the tissues of small hake, which in turn

show isotopic enrichment relative to their zooplankton prey. Small hake feeding on zooplankton and small fish are therefore probably secondary consumers.

5. CONCLUSIONS

$\delta^{13}C$ and $\delta^{15}N$ were used as indicators of the trophic level of the shallow-water Cape hake, *Merluccius capensis*. $\delta^{15}N$ appears to be a better indicator of hake trophic position than $\delta^{13}C$. This is consistent with the findings of Fry (1988), who concluded that $\delta^{15}N$ was a better trophic level indicator of organisms high up in the food web. Gut content analyses were used by Payne *et al.* (1987) to estimate the trophic position of hake. Small hake were found to consume mostly crustaceans, while the large fish were mainly piscivorous and sometimes cannibalistic. According to Ware (1992), hake are tertiary consumers. This study concludes that small and large hake appear to feed on different trophic levels, as the muscle tissue and bone collagen of large hake is enriched in $\delta^{15}N$ by 2-4$^{o}/_{oo}$ relative to small hake tissues. Small hake seem to be secondary consumers, having more positive $\delta^{13}C$ and $\delta^{15}N$ values relative to the zooplankton. Large hake are mainly tertiary consumers, being isotopically enriched relative to the small hake; although they occasionally feed on crustaceans, particularly on the west coast. Analysis of $\delta^{15}N$ in the Trophic Position Isotope Spectrum indicates that cannibalism may be more prevalent in *M.capensis* on the west coast than on the south coast. Hake on the south coast appear to feed on a wider variety of prey than on the west coast.

Sholto-Douglas (1992) found a significant difference in $\delta^{13}C$ in hake muscle from which the lipids had and had not been extracted. No experiments were carried out to determine whether $\delta^{15}N$ in hake tissues is affected by lipid extraction. As lipids do not contain nitrogen (Curtis 1983), it is unlikely that the outcome of this study would be affected by the fact that the lipids were extracted from the samples.

$\delta^{15}N$ on the west coast in muscle and bone collagen of all size hake was significantly higher than on the south coast. This may be due directly to the occurrence of upwelling on the west coast, and possible use of regenerated nitrogen by plankton on the south coast. These effects may be indirect, as the difference in $\delta^{15}N$ between the two coasts may be due to different

phytoplankton and zooplankton species composition. No significant difference was found in $\delta^{13}C$ between the west and south coasts.

$\delta^{13}C$ appears to be higher in zooplankton and the gut contents of small hake in February (summer) than in July (winter) in the vicinity of Cape Columbine on the west coast. This may be caused by the greater biological productivity associated with upwelling on the west coast. $\delta^{13}C$ was significantly lower in February than in July in the muscle tissue and bone collagen of small hake. This lag behind the zooplankton and gut content isotopic values may be due to the time taken for the dietary carbon to be integrated into the hake tissue. No seasonal difference was found in the tissues of large hake, possibly because the turnover time of the tissues is too slow to reflect changes over a period of six months. No seasonal variation was found in $\delta^{15}N$. However more frequent sampling is necessary to elucidate these results.

Hake sampled from Cape Columbine in July 1990 show $\delta^{13}C$ to be approximately $2^{o}/_{oo}$ higher in bone collagen than in muscle. This result is not observed when all samples are analysed together, as seasonal variations in the samples may have an effect and there is too much scatter in the data. Muscle tissue is enriched in $\delta^{15}N$ by approximately $2^{o}/_{oo}$ relative to bone collagen in all size fish, from both coasts. This may be due to different amino acid compositions of bone collagen and muscle, and the rates of integration of carbon and nitrogen into the tissues.

A positive correlation was found between $\delta^{15}N$ and hake length. This may be due to large hake feeding at a higher trophic level than small hake. The possible faster metabolic rate of small hake may enhance this effect. $\delta^{13}C$ values did not change with increasing fish length.

Ideas for further research arise from this study. If the basis for the isotopic differences between the west and south coasts is due to different nutrient types and concentrations, it would be necessary to sample the water on both coasts to test whether any isotopic differences exist. As $\delta^{13}C$ and $\delta^{15}N$ may change in the environment over a short period of time, isotopic analyses of different size classes of plankton sampled intensively over an upwelling cycle may show whether isotopic variability has a basis in physical or biological processes. The amino acid composition of hake muscle and bone collagen, and $\delta^{13}C$ and $\delta^{15}N$ values of dominant amino acids should be examined. A food web study tracing trophic enrichments using amino acids may prove interesting.

The biomass flow in a system is difficult to estimate, requiring intensive stomach content analyses and population estimates. It appears that the higher in a food web an animal is situated, the more complex the interactions within and between trophic levels become. It is therefore necessary to use as many appropriate techniques as are available to define trophic links. The use of stable isotope ratios, particularly $\delta^{15}N$, to complement existing analytical techniques may contribute to examining biomass flow in a system by providing integrated carbon and nitrogen pathways which are not as subject to bias as gut content analyses.

Stable carbon and nitrogen isotope ratios are a useful tool in the estimation of the long-term diet of hake integrated over the turnover time of the tissues analysed, which in the case of large hake appears to be longer than six months. Used in conjunction with gut content analyses, which may be used to estimate recent hake diet and specific species composition, this method allows the diet of an important commercial fish species to be studied more comprehensively. Additional information on the hake is shown by the isotope data, such as the absence of extensive alongshore migrations by *M.capensis*, and that hake on the south coast seem to feed on a wider range of prey species than on the west coast. As information on demersal fish such as hake is difficult to obtain easily and inexpensively, maximum use should be made of

available samples to determine as much as possible about the resource for effective management.

ACKNOWLEDGEMENTS

I thank my supervisors, Prof.J.G.Field and Dr J.A.Lee-Thorp for their assistance and encouragement during this project, their valuable discussions and comments on the manuscript. I thank the Director of Sea Fisheries Research Institute for permission to participate in research cruises on board F.R.S.*Africana* to collect samples. The assistance of members of the Demersal Section (S.F.R.I.) during these cruises is appreciated, and I would like to thank Dr S.C.Pillar, Mr D.W.Japp and Mrs S.E.Du Plessis for collecting samples when I was unable to be on board the ship. This work was undertaken in the Archaeometry Laboratory at the University of Cape Town. I thank Mr J. Lanham and Mrs D.Sholto-Douglas for technical assistance. I also thank Mrs D.Sholto-Douglas and Mr P.Monteiro for valuable discussions. The assistance of Mr C.Villacastin-Herrero with statistics and computer work is much appreciated. Last, and certainly not least, I would like to thank my parents, Neville and Joan Parkins for their consistent interest and encouragement.

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APPENDIX A - $\delta^{13}C$ AND $\delta^{15}N$ IN WEST COAST SAMPLES

$\delta^{13}C$ in hake samples

Date	Fish length (cm)	Muscle	Bone Collagen	Gut Contents
Cape Columbine				
July 1990	80	-14.05	-13.43	-14.59
	62	-14.99	-12.97	-15.45
	40	-14.97	-14.15	-15.20
	27	-15.28	-13.54	-14.50
	19	-15.27	-13.85	-17.11
	13	-15.51	-11.81	-17.54
May 1991	25	-15.14	-13.50	-15.15
	13	-16.87	-14.99	-16.70
	11	-15.80	-15.33	-16.47
Feb 1992	86	-15.59	-13.63	-15.62
	63	-15.41	-15.22	-15.82
	51	-16.39	-15.56	-17.00
	47	-14.94	-15.57	-15.64
	30	-16.00	-16.63	-17.77
	23	-16.30	-16.60	-17.81
Hondeklip Bay				
Jan 1991	62	-14.43	-12.47	-15.91
	48	-15.55	-13.24	-16.60
	34	-15.19	-14.17	-15.81
May 1991	20	-15.6	-14.08	-14.44
Cape Hangklip				
Jan 1991	46	-14.96	-12.84	-14.43
	37	-15.51	-16.67	-16.19

$\delta^{15}N$ in hake samples

Date	Fish length (cm)	Muscle	Bone Collagen	Gut Contents
Cape Columbine				
July 1990	80	17.05	16.08	15.70
	62	17.00	16.08	14.44
	40	15.59	14.16	
	27	14.57	13.67	14.72
	19	15.41	11.71	11.43
	13	12.84	13.68	10.48
May 1991	25	14.59	13.27	14.53
	13	13.69	12.23	12.84
	11	13.47	11.75	14.21
Feb 1992	86	17.18	14.81	14.61
	63	16.13	15.60	14.06
	51	15.42	14.97	12.75
	47	15.94	15.25	14.33
	30	14.04	12.87	11.66
	23	12.96	12.84	13.23
Hondeklip Bay				
Jan 1991	62	17.05	15.52	15.50
	48	16.48	14.84	14.86
	34	16.14	14.99	14.17
May 1991	20	14.15	13.16	12.63
Cape Hangklip				
Jan 1991	46	15.81	14.54	12.30
	37	15.05	14.15	14.06

$\delta^{13}C$ and $\delta^{15}N$ in zooplankton samples.

Date	Size (μm)	$\delta^{13}C$	$\delta^{15}N$
Cape Columbine			
July 1990	200-500	-18.17	7.19
	500-3500	-18.76	8.03
	>3500	-17.03	9.50
May 1991	200-500	-16.11	7.82
	500-3500	-15.42	8.11
	>3500	-17.19	10.08
February 1992	200-500	-17.09	9.57
	500-3500	-16.10	8.44
	>3500	-16.09	9.34
Hondeklip Bay			
May 1991	200-500	-16.11	9.53
	500-3500	-15.00	10.14
	>3500	-16.05	11.28

$\delta^{13}C$ and $\delta^{15}N$ in prey fish

Date	Species	$\delta^{13}C$	$\delta^{15}N$
Cape Columbine			
July 1990	*Etrumeus whiteheadi*	-16.06	14.02
	Coelorhynchus fasciatus	-14.48	16.22
	Sardinops sagax ocellatus	-16.37	12.92
May 1991	*Maurolicus muelleri*	-15.76	13.38
	Lampanyctodes hectoris	-14.93	15.28
Feb 1992	*Coelorhynchus fasciatus*	-16.09	16.53
	Helicolenus dactylopterus	-16.82	15.01
	Merluccius paradoxus	-16.21	14.09
	Trachurus trachurus capensis	-15.26	14.98
Hondeklip Bay			
Jan 1991	*Etrumeus whiteheadi*	-15.60	14.42
	Paracallionymus costatus	-15.65	14.07
	Maurolicus muelleri	-19.62	13.30
	Trachurus trachurus capensis	-14.79	14.55
	Merluccius paradoxus	-15.56	15.2
Cape Hangklip			
Jan 1991	*Cynoglossus zanzibarensis*	-17.18	14.48
	Sepia australis	-17.45	12.60
	Paracallionymus costatus	-17.10	13.93

APPENDIX B - $\delta^{13}C$ AND $\delta^{15}N$ IN SOUTH COAST SAMPLES

$\delta^{13}C$ in hake samples

Date	Fish length (cm)	Muscle	Bone Collagen	Gut Contents
Cape Infanta				
May 1991	50	-15.51	-13.43	-15.59
	29	-16.14	-14.69	-16.90
	17	-17.11	-15.45	-16.60
Feb 1992	84	-15.11	-12.26	-18.11
	60	-15.27	-15.23	-18.09
	39	-15.20		-16.84
	26	-16.69	-16.2	-17.35
	15	-18.66	-16.99	-19.93
Mossel Bay				
8 June 1991	92	-15.43	-13.93	-16.44
	75	-15.47	-14.66	-17.31
	55	-15.77	-15.33	-14.75
	39	-16.11	-15.94	-15.18
	26	-16.02	-15.97	-15.27
Plettenberg Bay				
June 1991	84	-14.86	-14.22	
	69	-15.27	-14.60	-15.66
	57	-15.51	-14.48	-16.13
	42	-16.11	-16.27	-16.05
	25	-16.72	-15.98	-17.30
	16	-15.33	-14.91	-16.25
Port Elizabeth				
June 1991	87	-14.79	-14.41	-15.01
	73	-15.06	-15.86	-16.60
	63	-15.42	-15.33	-16.86
	46	-15.58	-15.81	-15.44
	26	-14.19	-14.66	-16.51
	15	-15.28	-15.76	-17.04
Port Alfred				
June 1991	70	-15.06	-13.66	-19.41
	63	-14.81	-13.57	-15.88
	51	-15.15	-14.64	-15.14
	31	-15.06	-14.43	-16.54
	25	-15.07	-15.19	-15.46
	18	-15.36	-15.40	-16.54

$\delta^{15}N$ in hake samples

Date	Fish length (cm)	Muscle	Bone Collagen	Gut Contents
		Cape Infanta		
May 1991	50	15.30	13.91	13.14
	29	15.16	13.84	12.63
	17	14.37	13.03	14.56
Feb 1992	84	15.94	16.04	13.94
	60	16.18	14.27	13.37
	39	15.71		13.06
	26	14.39	12.84	10.07
	15	12.15	10.26	4.92
		Mossel Bay		
June 1991	92	17.01	13.87	13.87
	75	15.84	14.61	14.44
	55	15.30	14.16	12.68
	39	14.46	13.95	12.81
	26	14.11	13.89	13.65
		Plettenberg Bay		
June 1991	84	17.45	15.65	
	69	16.06	14.16	14.7
	57	15.55	14.13	13.64
	42	13.80	12.37	12.04
	25	13.83	13.08	11.52
	16	11.59	10.44	9.54
		Port Elizabeth		
June 1991	87	15.54	14.62	15.03
	73	15.56	15.56	14.07
	63	14.70	13.22	13.45
	46	14.14	13.78	12.66
	26	12.00	11.19	10.4
	15	11.84	10.10	8.41
		Port Alfred		
June 1991	70	17.45	12.64	10.93
	63	16.06	12.47	13.05
	51	15.55	12.51	11.21
	31	13.80	11.67	8.67
	25	13.83	10.49	10.54
	18	11.59	9.53	8.61

$\delta^{13}C$ and $\delta^{15}N$ in prey fish

Date	Species	$\delta^{13}C$	$\delta^{15}N$
Cape Infanta			
May 1991	*Etrumeus whiteheadi*	-16.25	13.38
	Engraulis capensis	-16.16	13.27
Feb 1992	*Trachurus trachurus capensis*	-17.34	13.58
	Etrumeus whiteheadi	-17.19	12.85
Mossel Bay			
June 1991	*Etrumeus whiteheadi*	-16.66	14.02
Plettenberg Bay			
June 1991	*Etrumeus whiteheadi*	-16.42	13.14
	Trachurus trachurus capensis	-16.55	13.83
Port Elizabeth			
June 1991	*Etrumeus whiteheadi*	-16.76	12.57
	Trachurus trachurus capensis	-16.25	13.53